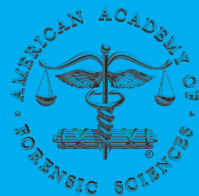


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*72<sup>nd</sup> Annual Scientific Meeting  
Anaheim, CA  
February 17-22, 2020*



## AMERICAN ACADEMY OF FORENSIC SCIENCES

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### ***PROCEEDINGS***

of the American Academy of Forensic Sciences 72<sup>nd</sup> Annual Scientific Meeting

The *Proceedings* of the American Academy of Forensic Sciences is an official publication of the American Academy of Forensic Sciences (AAFS). It is devoted to the publication of the abstracts of technical oral papers and posters presented at the AAFS Annual Scientific Meeting. These include various branches of the forensic sciences such as anthropology, criminalistics, digital evidence, engineering, immunology, jurisprudence, odontology, pathology, psychiatry, questioned documents, and toxicology. Similar submissions dealing with forensic-oriented aspects of the social sciences are also included.

Please note that some of the abstracts included in the *Proceedings* deal with topics, results, and/or conclusions that are controversial. The publication of abstracts does not imply that the AAFS, its sections, or the individual section program chairs/committee members have verified or agree with the studies, results, and/or conclusions of each abstract. During the process of planning a scientific program, it is impossible to “peer-review” each abstract and presentation to the degree that is accomplished during manuscript review. Abstracts and presentations are accepted, in part, so they can be critiqued and reviewed by other scientists. Thus, a forum is created to discuss controversial issues.

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# ***PROCEEDINGS***

**of the American Academy of Forensic Sciences**

**February 2020**

**Volume XXVI**

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### S1 Interdisciplinary Symposium: Progress in the Forensic Sciences Since the 2009 National Academy of Sciences (NAS) Report

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**Learning Overview:** Attendees will have an appreciation of the impact the 2009 Report by the NAS entitled *Strengthening Forensic Science in the United States: A Path Forward* has had on several forensic disciplines.

**Impact on the Forensic Science Community:** Forensic sciences have been under the shadow of the NAS Report for the past ten years. This symposium will impact the forensic science community by providing an update on the state of many disciplines with respect to method reliability and testimonial boundaries, both of which were called into question by the NAS Report.

The NAS Report, *Strengthening Forensic Science in the United States: A Path Forward*, commonly referred to as the NAS Report by the forensic community, was released in February 2009 during the Annual Scientific Meeting in Denver, CO. This Report was unprecedented because it brought to light many issues that had been hampering the evolution of forensic science. It provided 13 comprehensive recommendations, starting with the establishment of a National Institute of Forensic Sciences (NIFS). This was described as an independent federal entity designated with the responsibility of supporting and overseeing forensic science across the country. The authors advocated that law enforcement agencies should no longer be the primary administrators of their crime laboratories and that medical examiner systems should replace coroner's offices. Laboratory accreditation, individual examiner certification, and standardization of procedures were additional recommendations proposed in the NAS Report. Two significant recommendations to the forensic sciences, more specifically to the pattern-based evidence disciplines, revolved around: (1) improving their scientific foundations and reliability through research and validation, and (2) ensuring the accuracy of testimony offered by experts in court. Many of the NAS recommendations have not come to fruition, including the creation of NIFS. However, changes have occurred in response to some of the recommendations. Various questions can be posed to assess the impact of those changes. Has the community devoted time and resources to validating current techniques and methods? Are new disciplines embracing the scientific method? What does a "match" or an "identification" mean today? How conclusively are examiners allowed to state their results? What does the future hold for forensic expert testimony? Have court systems outside of the United States taken any notice of the changes occurring here?

This year's symposium will feature 13 speakers across both scientific and national borders who will provide their perspectives on how the NAS Report has changed (or not) their business practices over the past decade. Many of the forensic disciplines discussed in the report will be addressed, including DNA, friction ridge, firearms, documents, odontology, and digital/multimedia evidence examination. Responses in crime scene analysis and medicolegal investigations will be presented as well. Members of the legal community will also share their thoughts on how they interpret the conclusions made by forensic experts in written reports and in trial. In addition, the efforts of the National Institute of Justice (NIJ) to fund research in these areas will be discussed. This symposium intends to be a valuable session to forensic science practitioners, managers, and legal representatives.

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#### National Academy of Sciences, Validation, Testimony



### S2 YFSF: 25 Years of Crossing Borders: Young Forensic Scientists and Their Collaborations

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**Learning Overview:** After attending this presentation, attendees will better understand forensic science and how everyone's work can impact and enhance other scientists, and vice versa, regardless of the discipline. In addition, attendees will learn about the advantages and importance of proper resume writing and interviewing skills.

**Impact on the Forensic Science Community:** The Young Forensic Scientists Forum (YFSF) has historically provided a venue for those forensic scientists with less than five years of professional experience to interact with, and further integrate into, their prospective fields, as well as to learn about all the other disciplines. The YFSF should provide the space and opportunity for attendees/speakers to grow, develop, network, and learn from one another. This will allow young scientists the confidence to progress in their fields and one day hopefully provide the same opportunity to another young scientist.

Every year, the American Academy of Forensic Sciences (AAFS) draws in thousands of globally recognized experts and professionals to present research, hold workshops, and showcase the various disciplines represented within the Academy. Further, the YFSF offers two opportunities for young forensic scientists to present their own work or research: the Bring Your Own Posters (BYOP) Session and the Bring Your Own Slides (BYOS) Session.

Currently celebrating its 25<sup>th</sup> anniversary, the YFSF presents for the 72<sup>nd</sup> Annual Scientific Meeting in Anaheim, CA, *25 Years of Crossing Borders: Young Forensic Scientists and Their Collaborations*. This Special Session will be held on Tuesday, February 18, 2020, and will feature speakers from many of the AAFS sections who will discuss their work and how they crossed borders, figuratively and literally, to collaborate with other disciplines. Through these presentations, attendees will learn how experienced practitioners and professionals have contributed to forensic science as a whole and how their work has benefitted from and was helped by interdisciplinary combining of knowledge bases. With a rapid push for specialization within a given discipline, it has remained vital for leaders in forensic science to remember the importance of cross-discipline collaboration and partnership. Through this Special Session, forensic experts will provide mentorship to young forensic scientists by inspiring interdisciplinary cooperation in casework, research, and community engagement.

Finally, in the spirit of interdisciplinary collaboration and to honor our commitment to improving and advancing communication, for the first time, the YFSF is seeking to provide the opportunity to attendees to network with AAFS members and many others to encourage the sharing of information and experiences. This year's Special Session has been re-designed to incorporate a networking morning coffee break where all YFSF attendees and distinguished members from the AAFS sections can meet, mingle, and have discussions. Further, the discussion and resume review panel has been lengthened and will be enhanced by the addition of a speaker specifically addressing resumes and interview preparation, so don't forget to update and bring several copies of your resume with you!

#### YFSF, Research, Education



### BS1 Ethno-Cultural and Religious Considerations in the Management of the Dead

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**Learning Overview:** After attending this presentation, attendees will have a greater knowledge of the ethics, customs, norms, and taboos surrounding death and the dead for many minority cultures and religions within their professional and personal communities. This presentation will describe several cultures and religions that forensic professionals may encounter in their jurisdictions, both in the United States and worldwide, along with each respective approach to the management of their dead, based upon this better understanding.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving to assist the forensic professional in overcoming some of the obstacles that are present when identifying, investigating, and examining a decedent that is a member of these ethno-cultural communities.

Culture and religion profoundly influence many aspects of modern life. These factors also carry forward after death and may affect our management of the deceased. It is recognized that increasing human migration across global geographical borders results in decedents sometimes being managed within a culture and community that is foreign to them. Loved ones, family, and members of the integrated community of the deceased also bring expectations, norms, and taboos to a forensic setting and these influences may at times increase the complexity of and even hinder the process of examination, identification, and release of the dead. Although it is impossible to be intimately familiar with all religions and cultures, we as forensic professionals should be educated and sensitive to the roles each may play in our respective areas of expertise while avoiding the risk of stereotyping the deceased.

It is important to the process and to the outcome that the forensic professional understands not only the ethno-cultural and religious considerations associated with the deceased, and other members of the affected community, but also their own, and how these may relate to the context and perhaps to any biases that may be subsequently introduced. So, the complexity of the juxtaposition of different cultures and expectations may be multiplied in a situation where the forensic practitioner is anything other than the ideal of absolute neutrality.

The interplay of the deceased's culture and the forensic professional's legal and ethical responsibilities can pose significant challenges when experienced against a backdrop of factors including gender and acculturation, notably in cases in which there are multiple and/or commingled fatalities being examined at one time in one spatial setting. Varied cultural and religious mores for managing the dead also dictate appropriate disclosures, privacy, and communication with the relatives of the deceased. Some difficulties can, at least, be partially overcome with an understanding of these sensitivities.

This presentation hopes to highlight many of the unique aspects of the belief systems and social constructs that may be encountered during the career of a forensic professional in the context of the management of the dead and provide tools and a framework for increased sensitivity and understanding. This, in turn, will provide an opportunity to allow the dignity and basic human rights of the deceased to perhaps be respected more fully.

Examples will be drawn from many cultural and religious backgrounds, including Islam, Judaism, Christianity, Hinduism, Buddhism, Native American, Maori, and Pasifika peoples.

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### Religion, Culture, Management of the Dead



### BS2 The Working Stiffs: Writing and Publishing the Experiences of a Forensic Pathologist in Both Fiction and Non-Fiction

*Judy Melinek, MD\*, PathologyExpert, Inc, San Francisco, CA 94121; T.J. Mitchell, BA\*, PathologyExpert Inc, San Francisco, CA 94121*

**Learning Overview:** The goal of this presentation is to demonstrate how a collaboration between a scientist and a writer can effectuate the communication of complex forensic concepts to a lay audience.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping attendees be able to communicate scientific concepts to a lay audience, in writing and in testimony, and by navigating issues pertaining to the confidentiality of medical information.

The thesis of this presentation is the importance of reflecting scientific rigor in writing both non-fiction and fiction for a lay audience. The challenges of writing while continuing to testify as an expert witness in real-life criminal and civil court cases will be explored. In addition, the perpetual tension between fiction and science—maintaining a balance between narrative demands and medical verisimilitude—will be discussed.

Journal writing can be an effective way of documenting what a scientist encounters in clinical detail. Other ways of tracking forensic cases include spreadsheets and medical databases. In some offices, it is not uncommon to collect newspaper clippings of the deaths that are investigated and picked up by the media and place them in the case file. However, newspaper accounts may not be accurate and examples will be presented of how eyewitness statements and assumptions made by the police or death investigators can be found to be in error when the body is taken to autopsy. Writing and documenting what a pathologist sees at the time of autopsy is crucial for clinical decision making and peer review, including review by outside experts. Furthermore, taking written notes about one's daily experiences and what one learns through those experiences can also become a psychological outlet: a way to “decompress” after a long day of working in extreme conditions, or a way to deal with the grief and anger of family members.<sup>1,2</sup> Writing can be a therapeutic exercise that supplements formal training in forensic pathology and should be encouraged in forensic fellowship training.<sup>3</sup>

Working in an understaffed and underfunded environment can also put stress on the forensic team and may incubate intramural tension. Mass fatality response, along with a backlog of routine cases delayed by ancillary lab studies, can add to the pressures pathologists experience. Journaling can help forensic professionals deal with the stresses of the job, but non-disclosure agreements and privacy policies may restrict professional communication and publication in a public forum. In academic writing, de-identification is used when sharing case reports, and this method can be used in writing non-fiction for public consumption. Fictionalizing actual forensic cases can allow scientists with a way to explore the work they do and share their experiences, both with other professionals and with the lay public.

A case report will be presented of a petty thief who stole a laptop and was chased down and shot dead in public by its owner. This case involved a complex gunshot wound trajectory analysis and the collection of property as evidence. Forensic-noir mystery writers, such as Kathy Reichs, Patricia Cornwell, and Jon Jefferson and Dr. William Bass, all of whom worked in various capacities in the forensic sciences professional discipline, inspired this presentation to explore ways to educate the public about real-world forensic science through the use of a fictional narrative voice.

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2. Cognitive processing, disclosure, and health: Psychological and physiological mechanisms. Susan K Lutgendorf, Philip Ullrich. American Psychological Association, 2002
3. Shannon L Arntfield, Kristen Slesar, Jennifer Dickson, Rita Charon. Narrative medicine as a means of training medical students toward residency competencies.

#### Forensic, Pathology, Writing



### BS3 The Science Behind Traffic Accident Reconstruction With Real-World Crashes

*Kurt D. Weiss, MS\*, Case Study Collision Science, LLC, Santa Barbara, CA 93190; Darren Franck, MSME, Advanced Engineering Associates, Inc, Charleston, WV 25304*

**Learning Overview:** The goal of this presentation is to share with the forensic science community the data sources and detailed methodology used in traffic collision reconstruction.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating through real-world case studies the data used and methodologies applied to the reconstruction of traffic collisions.

An analysis of four real-world crashes will demonstrate the science behind traffic accident reconstruction. Reconstructing a crash is a multidisciplinary process. The reconstructionist gathers objective and subjective information, performs case-specific research, then applies the fundamental principles of physics to determine, for example, impact classifications, vehicle heading, impact configuration, impact (closing) speed, velocity change or Delta-V (DV), rollover distance, roll count, and roll rate. The reconstruction results are important input to the injury analysis and the assessment of injury patterns, risk, and prevention.

The primary information source is the Traffic Collision Report, which may provide: (1) objective party information (e.g., occupant and vehicle year, make, model, Vehicle Identification Number (VIN)); (2) objective site information (location and roadway, intersection, and Global Positioning System (GPS) details), scene diagrams with measurements of physical evidence (tire friction marks, fractured glass, roadway scrapes and gouges, scarred tree bark, fractured signs or light posts, disturbed dirt and/or vegetation), point of impact and vehicle rest locations; (3) at-scene police photographs of site evidence and vehicle damage; and (4) subjective information (e.g., party statements, vehicle code violations, police collision summary and primary collision factor identifying party and cause).

Case-specific research may include: (1) at-scene witness photographs, which are often taken with cell phone cameras; these photographs are crucial absent police photographic documentation of site and vehicle evidence; (2) security videos that help refine the analysis by providing objective evidence of vehicle movements prior to and after the crash; (3) aerial photography from online image archives that orient and supplement scene diagrams; (4) drive-through videos that provide a street view of the roadway and/or intersection; (5) site inspections that clarify roadways, controls, and obstacles; (6) subject vehicle inspections with crush profile measurements that document property damage and reveal relative impact alignment or vehicle movement; (7) Airbag Control Module (ACM) data that quantify pre-crash vehicle speeds and accelerations, and driver actions (e.g., swerving, braking); (8) subjective information (e.g., witness statements and deposition testimony) that help qualify collision events and estimates of vehicle speeds, distances, and movements; these sources may also provide prior useful site, vehicle, or occupant history; and (9) exemplar vehicle inspections, scaled vehicle drawings, specifications, and crash test data that may be used to determine the collision severity.

An analysis of four real-world crashes demonstrate the science behind traffic accident reconstruction.

**Case 1**—fatal collision: vehicle crush measurements quantify collision severity.

**Case 2**—vehicle-motorcycle crash: download of ACM yields pre-crash data.

**Case 3**—wrong-way, intoxicated driver in a high-speed crash: patrol car's on-board video quantifies vehicle impact speeds.

**Case 4**—vehicle-motorcycle crash: stationary security videos quantify vehicle impact speeds.

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#### Traffic Accident Reconstruction, Data Sources, Real-World Crashes





### BS4 A Tour of a Cannabis Extract Manufacturing Facility in Maine

*Jennifer A. Paris, BS\*, New Hampshire State Police, Concord, NH 03305; Anna K. Weaver, BS, Concord, NH 03305*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the operation of a small state-licensed and inspected cannabis extract manufacturing facility.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by promoting an understanding of the processes used to concentrate cannabinoids, how products are diluted, prepared, and packaged in order to comply with the relevant laws and regulations, and may be helpful for attendees who are assisting in building a local or state jurisdiction's medical or recreational marijuana program.

The state of Maine enacted a medical marijuana law after a citizen-led ballot initiative passed in November 1999. Since that point in time, multiple amendments have been made to the statute following feedback from patients, caregivers, medical providers, and a specially developed task force. Although a 2016 law was passed to allow for the recreational use of marijuana, and a 2017 law was passed concerning taxation and other regulations surrounding recreational marijuana, the commercial sale of the plant and products made from it have not yet begun.

Today, Maine permits both growing and processing of marijuana by individuals holding a medical card (i.e., "patients"). In addition, patients and caregivers are allowed to use a third-party manufacturing facility to convert their harvested marijuana or hemp into concentrated forms. These facilities are registered, inspected, and licensed by the state of Maine. Patients and caregivers are expected to adhere to strict procedures regarding site safety and security, the quantity and type of plants on site, the quantity of harvested product on site, the use of "inherently hazardous substances," and the disposal of waste materials, among other standards.

This presentation will briefly cover the regulations in place for Maine's program, then focus on the specifics with respect to a small, registered manufacturing facility. Chemists from the New Hampshire State Police Forensic Laboratory were given the opportunity to tour one such facility in the summer of 2019 while extractions were in progress. This cannabis extract manufacturing facility employs super critical carbon dioxide processing of harvested marijuana and/or flowering hemp grown by patients or their caregivers to produce highly purified concentrates devoid of terpenes. In addition, the facility was equipped with a closed-loop extraction system to produce butane honey oil, shatter, and "diamonds." Discussions regarding purity, concentration, cannabinoid profiles, and all aspects of the operation, including the evolution of the facility's practices over time, took place with the owner/operator of the facility. Attendees will be shown the outdoor grow area, extraction and purification processes, preparation of food products, and product packaging materials at the visited facility.

Given the increased number of states where cannabis products are legal for medical or recreational use, it is important for those involved in the analysis of seized drugs to have an understanding of the processes used to concentrate cannabinoids from harvested marijuana and flowering hemp. Furthermore, it is helpful to be familiar with how products must be diluted, prepared, and packaged in order to comply with relevant laws and regulations.

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### Cannabis, Extract, Manufacturing



### BS5 Practical Aspects of Developing and Incorporating a Postmortem Computed Tomography (PMCT) Service Into a Medical Examiner's Office

*Lauren Edelman, MD\**, Travis County Medical Examiner, Austin, TX 78724; *Keith Pinckard, MD, PhD*, Travis County Medical Examiner, Austin, TX 78724

**Learning Overview:** After attending this presentation, attendees will understand the concepts and considerations involved in incorporating PMCT into the practice of a medium-sized, regional medical examiner's office, including staffing, scanning protocols, triage of cases, study interpretation, and novel and alternative data storage solutions. Developing and incorporating a PMCT service is a complex project, with broad implications for many aspects of an individual office.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a framework and blueprint that can be used to incorporate and implement this burgeoning technology within the scope of death investigation for medical examiner's and coroner's offices.

The services of a consultant were engaged as part of the budget request associated with purchasing a Computed Tomography (CT) scanner. The consultant provided guidance for technical and structural considerations critical for drafting the purchasing bid documents and review of the bids for the purchase of the CT scanner and associated equipment. After installation by the vendor, the consultant also provided the basic scanning protocols that are programmed into the scanner for ease of use and include protocols for standard adult, bariatric, pediatric, charred remains, skeletal remains, and decomposed remains. This is a key component, since standard protocols that are provided by CT scanner vendors are designed for clinically based studies on living patients.

As part of the purchasing contract, forensic autopsy technicians, none of whom had formal radiology technology training, received in-person instruction in the practical aspects of operating the equipment. Performing the scans with the appropriate protocols requires minimal direct management by the forensic autopsy technicians. Once the protocols are in place and validated, scanning proved to be relatively simple and straightforward. Scanning is performed during routine working hours on a rolling basis as decedents are brought into the office; scans are completed prior to the processing and external examination of the body. All decedents who are physically capable of fitting into the CT scanner are imaged. The rationale for this is two-fold. First, scanning every decedent as a matter of routine may guide how the pathologist approaches the case. Second, scanning creates a permanent 3D radiographic record of the body that can be reviewed long after the body has been permanently altered by autopsy.

The scans are reviewed and interpreted by the forensic pathologists as a group during a morning conference prior to performing examinations and again individually when medical examiner reports are written. The medical examiners in the office received training in CT interpretation by two physicians with prior PMCT experience who were already on staff. The learning curve of CT interpretation is very steep; familiarity with visualizing cross-sectional anatomy in three dimensions simultaneously comes surprising quickly. Furthermore, because all decedents are scanned, each pathologist gets immediate feedback by comparing autopsy findings to what was observed on the scans—a practice that rapidly builds interpretive skills and confidence. Once the medical examiner staff is comfortable with reading CT scans, decisions can be made regarding which types of cases may be able to be supplanted by PMCT.

A novel cloud-based storage and image-viewing software solution was purchased rather than a traditional Picture Archiving and Communication System (PACS). It has virtually identical capabilities, including permanent long-term storage with system redundancy, at a fraction of the cost of a standard PACS solution. Per this research, PACS is a vastly more sophisticated and ultimately far more expensive solution than what is actually required for death investigation purposes. For offices affiliated with medical centers, it may be possible and cost-effective to "partner" and obtain a node on a hospital's PACS. However, offices without this benefit have struggled to devise a system tailored to the unique needs of death investigation without the associated burdensome cost of a PACS—a cost factor that may deter an office from obtaining PMCT capability altogether. Per this research, this is the first office to pilot and utilize this type of storage and viewing solution.

As more death investigation offices around the country begin to integrate CT imaging as part of the physician's toolkit, it is critical to share operating procedures and innovative solutions to common problems to assist others in participating in this exciting advancement in the field of forensic pathology.

#### Postmortem Imaging, Computed Tomography, PACS



### BS6 Is a Soldier's Combat Stress a Reason to Commit Five Murders?

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of locating, identifying, and collecting evidence and the documentation of the crime scene associated with a mass casualty investigation. Attendees will also be aware of the policies and procedures for conducting a crime scene investigation (timeliness, thoroughness, and timely reporting) and casualty liaison briefings provided to the primary and secondary next-of-kin. This presentation will also enable attendees to understand how the psychological evaluation of the subject may or may not have affected the outcome of the trial by court-martial and the results of the court-martial.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that a mass casualty investigation must be conducted in a timely and thorough manner in order to provide the court with the best evidence for conviction. The psychological evaluation will assist in determining if the subject was mentally responsible, and, if so, whether he should receive life with or without parole or the death penalty.

SGT John M. Russell had been seen at the Combat Stress Center (CSC), Camp Liberty, Iraq, on three separate occasions and had a noon appointment on May 11, 2009. At approximately 1:41 p.m., on May 11, 2009, the United States Army Criminal Investigation Command (CID) was notified of the shooting of several personnel inside the CSC. The investigation revealed that at approximately 12:30 p.m. on May 11, 2009, SGT Russell, Camp Stryker, Iraq, entered the CSC and methodically shot and killed five United States Service Members (a Navy Commander and an Army Major, a Sergeant, and two Private First Classes). SGT Russell was also listed for the offense of attempted murder and aggravated assault when he fired at two soldiers and missed. The Navy Commander was the only victim known to SGT Russell. SGT Russell, who did not have a weapon, gained possession of his escort's weapon (an M16 rifle), ordered his escort from the vehicle, then drove back to the CSC where he opened fire on unarmed personnel. Due to the regulatory requirements, all soldiers were required to carry their assigned weapons at all times in the combat environment on most camps; the only exception was a medical facility. Soldiers entering the CSC were required to place their weapons in an arms room while being treated; therefore, all soldiers in the Combat Stress Center were unarmed and vulnerable. SGT Russell was subsequently disarmed and placed under apprehension.

More than 28 CID special agents in Iraq were involved in processing the crime scene and interviewing the victims, witnesses and subjects. More CID special agents around the world were involved in the autopsies, background investigation of the subject, and developing information for the court-martial. The preliminary crime scene reconstruction took about one week (the crime scene was maintained for many years, until it had to be bulldozed). The special agents conducted bloodstain spatter analysis, ballistic reconstruction, and a plethora of other forensic analyses of the five separate areas where the victims were murdered.

SGT Russell was evaluated and determined not mentally competent to stand trial, so SGT Russell was placed in a mental health facility where he was evaluated for approximately four years. During this time, he was seen by several mental health professionals, questioned to determine his mental status, and medicated until he was considered restored to competency and returned to military custody.

On May 15, 2012, prosecutors decided to seek the death penalty and SGT Russell's defense stated he would pursue an insanity defense. On May 18, 2012, I Corps, Joint Base Lewis-McChord (JBLM) released the General Court-Martial Convening Authority (GCMCA) findings. The GCMCA referred court-martial charges against SGT Russell to a General Court-Martial empowered to adjudge a capital sentence.

During the court-martial proceedings, the defense blamed the combat mental health professionals for SGT Russell's actions and indicated that they could have prevented this mass casualty. SGT Russell was subsequently found guilty and on May 16, 2013; an Army judge sentenced SGT Russell to life without the possibility of parole for the shooting death of two care providers, two patients, and an escort. The judge stated "You are not a monster, but you have knowingly and deliberately done incredibly monstrous things."

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### Murder, Investigation, Stress



### L1 The Disappearance and Murder of Sierra LaMar: A Multidisciplinary Case Study

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**Learning Overview:** The goal of this presentation is to inform attendees of a complex homicide investigation that was heavily fueled by physical evidence examinations, including DNA, trace evidence, digital and video evidence, latent prints, and questioned documents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showcasing the ability for multiple forensic disciplines to work together to achieve the maximum potential of the evidence items submitted in a missing person case turned homicide.

Fifteen-year-old Sierra LaMar left her Morgan Hill, CA, home on March 16, 2012, but never made it to school. Her school bag, containing the clothes she'd been wearing the day she disappeared, and her discarded cell phone were found a short time later. Thus began an investigation that utilized multiple crime lab disciplines, culminating in a months-long 2017 trial that found Antolin Garcia Torres guilty of kidnap and murder, even though Sierra's body has never been found.

DNA was instrumental in identifying a suspect. Biological evidence found on Sierra's clothes matched to Garcia Torres in the Combined DNA Index System (CODIS), and a later search of his vehicle yielded additional evidence. The DNA testing for this case, much of which was developed in the first few days following Sierra's disappearance, utilized a plethora of DNA techniques and resulted in a variety of conclusions, each of which required a different type of statistical analysis, including mixtures, paternity testing, and Y-chromosomal Short Tandem Repeats (Y-STRs).

Trace evidence played a big part in this case. The trace evidence analyses required travel to assorted sites in Morgan Hill to collect comparison samples for testing. Examinations of Sierra's clothing and Garcia Torres' vehicle revealed various types of trace evidence, including hair, fibers, glass beads, soil, botanicals, and insect parts. Extensive examinations were performed on Sierra's cell phone, which had turned on and off several times following her disappearance. This prompted a visit to the Samsung® facility in South Korea by an investigator involved in the case.

Questioned documents examination was performed when a notebook of Sierra's was discovered at school, with notes written in it suggesting she was thinking of running away. Video evidence was paramount in this case. At the beginning of the investigation, video evidence from near the school bus stop and from the school bus itself was reviewed to see if Sierra had made it that far that morning. Later, video from the parking lot of the defendant's home and from his transactions at a bank were converted to a format that could be more easily viewed in court. In addition, video showed the defendant a few nights before Sierra's disappearance purchasing only a gallon of bleach and a turkey baster—a strange purchase for a 21-year-old male to be making by himself at night.

Prior to the abduction of Sierra, there were multiple attempted kidnappings in Morgan Hill. Investigators, interested to see if they could somehow link these attacks to Sierra's disappearance, requested that a Taser®, left at one of the scenes by the perpetrator, be processed for latent prints. A latent fingerprint was developed on the battery of the Taser® and was identified as Antolin Garcia Torres' print. Therefore, these attempted kidnappings became a part of the Sierra LaMar trial, showing that the defendant had a history of attacking women, and he was found guilty of these crimes as well. The work conducted by all these forensic disciplines in conjunction helped find a suspect, connected him to other crimes, and put to rest several defense theories. Antolin Garcia Torres was sentenced to life in prison without the possibility of parole.

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**Multidisciplinary, DNA/CODIS Hit, Trace Evidence**



### L2 Fingerprinting the Brain: Mind, Memories, and Malingering

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**Learning Overview:** After attending this presentation, attendees will better understand the role of brain fingerprinting to detect concealed information in forensic criminal cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing the competence of attendees through increasing the ability to interpret an emerging scientific method used to detect concealed information stored in the brain via the use of Electroencephalographic (EEG) brain responses/brainwaves.

Between 10% and 70% of criminal defendants claim crime-related amnesia; there is limited published literature on objective assessments of memory loss in a criminal setting. Interview techniques combined with psychological testing have been developed to assess possible malingering of memory loss. Investigators have long sought a reliable means of detecting deception. The Guilty Knowledge Test (GKT) is an early predecessor to brain fingerprinting and measures the individual's autonomic response to information known or not known about a crime.<sup>1</sup>

Recently, brain fingerprinting techniques using brain wave response have emerged as a scientific method to detect potentially concealed information stored in the brain. Brain fingerprinting techniques measure EEG brain responses/brainwaves and compute a determination of "information present" (the subject knows the critical information) or "information absent" (the subject does not).<sup>1</sup> According to this theory, if the individual is familiar with the information presented, a P300 brainwave is emitted and measured by an EEG.<sup>2</sup>

Dr. Farwell reports that in more than 200 test cases, brain fingerprinting yielded a 100% accuracy rate with a 0% error rate in distinguishing those who possessed specific target knowledge from those who did not.<sup>1</sup> As an example, in an experiment on 17 Federal Bureau of Investigation (FBI) agents, analysis using this P-300 brain fingerprinting technique yielded a 100% identification rate of FBI agents with FBI-relevant knowledge.<sup>1</sup> Dr. Farwell has coined the term "Farwell Brain Fingerprinting" (FBF) that he proposes has the ability to detect concealed information.<sup>1</sup>

The application of this FBF technique to forensic criminal cases was highlighted in the 2003 case of *Harrington v. State*.<sup>3</sup> Terry Harrington sought to overturn his 1978 murder conviction on several grounds, including new evidence in the form of FBF that was not available at his original trial. Dr. Farwell administered his test to Mr. Harrington and rendered a report to the Iowa District Court analyzing Mr. Harrington's responses to information about the crime. Dr. Farwell asserted that his analyses supported Mr. Harrington's assertion that he was not guilty and, therefore, it should be allowed into evidence to help overturn his conviction. In contrast, the district attorney challenged these results, noting several factors that could have impacted the FBF outcome. The panel will review how the admissibility of this newly emerging FBF technique was addressed by the district and appellate courts and its impact, if any, on the legal outcome of Mr. Harrington.<sup>4</sup> The strengths and limitations of this approach will be discussed and admissibility under the *Daubert* standard will be highlighted.

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#### Brain Fingerprinting, Lie Detection, Malingering



### ES1 Dracula, Twilight, and Blood Cults: Why Is It That Vampires Never Die?

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**Learning Overview:** After attending this presentations, attendees will: (1) gain knowledge in the historical, medical, and psychoanalytic theories behind vampire beliefs; (2) advance skills regarding the manner in which “vampirism,” “vampire cults,” and “vampire communities” may present clinically and forensically; and (3) improve knowledge in historical reports of sadistic mass murder that may have contributed to vampire lore.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the existing knowledge of the fundamentals of the current field and thus improve forensic competence and performance.

From the ancient world to the present, vampires have held a tight grip on our fascination and fear and this has yet to be released. Traces of the legend are found in some of the oldest surviving mythologies and have spilled over into history and lore. Such mythology associated vampires with female figures representing potential destruction and with children in a state of dependency and helplessness. Similar beliefs are traced to ancient cultures across the globe, often depicting demonic female figures who fused relationships between the living and the dead, expressed through blood rituals as well as sexualized and aggressive exchanges.<sup>1</sup>

However, it was Bram Stoker who introduced us to the great Dracula in the late 19<sup>th</sup> century, and it was this quintessential Byronic hero who fathered countless other characters. From the monstrous presentations of Nosferatu, and Underworld’s Markus, to the charming Lestat and adorable sparkly Edward, and everything in between, our fascination with Dracula and his ever-evolving progeny shows no signs of decay.

In the modern era, some vampires have been transformed from evil beasts to misunderstood, and ostracized, supernatural heroes.<sup>2</sup> With this change, some of their ideologies went from that of those cursed with tormented “un-deadness” to those having (and sometimes offering) immortality; from forced isolation to offering a new community; from subhuman viciousness to displaying animal traits that convey energy and natural instincts. Flaunting the appeal of the aristocrat or outlaw and inciting adventure at once terrifying and safe, the vampires in these stories have “evolved” into uncannily irresistible figures.<sup>3</sup> What is it that so intrigues us about them? This presentation will lead us on a journey where we can explore this fascination via culture and psychoanalytic theory, as well as historical figures purported to have inspired the legend—tales of which include sadism and mass murder eclipsing any terror found in fiction.

Also discussed will be cases from the early 1800s where the misperception of infectious illness caused mass hysteria, leading to the belief that the dead were rising from their graves and literally sucking vitality out of the living. This hysteria ultimately culminated in the cannibalistic rituals of the recently deceased to stop the dead from harming the living. However, such a ritual has been documented as recently as 2003, proving that these seemingly old beliefs from a forgotten time of darkness and irrational superstition for some reason draw appeal to this day.

Indeed, we are far from immune to such seeming madness. Modern vampire “cults” tragically show us how an obsession with these creatures can go too far. What drives such people into these alternate realities? Mental illness? Sadism? Or is there more complexity to these stories? Perhaps we will never know, but one thing is certain: while legends postulate many ways that vampires may be killed, time has proven that they will, indeed, live forever.

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#### Mass Murder, Blood Cults, Vampire



### W1 Drug Delivery Homicide: Prosecution, Defense, and Expert Testimony

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**Learning Overview:** The goals of this presentation are to: (1) discuss the theories of prosecution and defense under “drug-delivery-resulting-in-death” statutes and sentencing; (2) describe and explain the nuances of the Supreme Court of the United States (SCOTUS) decision in *Burrage v. United States*; (3) analyze the strengths and weaknesses of fact patterns drug-delivery-resulting-in-death cases; (4) identify and articulate the limitations of toxicological and medical testimony in these cases; and (5) prepare for testimony and direct and cross-examination in drug-delivery-resulting-in-death cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing background on the legal challenges for charging, defending, or providing opinions and testimony in cases in which an individual's death is alleged to have resulted from another individual's actions in supplying the drugs that caused the death. Prosecutors, defense attorneys, and testifying experts will be better prepared to provide scientifically supported evidence in the appropriate context to triers of fact in these cases.

With the advent of the opioid crisis, in addition to traditional prosecutions for drug possession and trafficking, there has been an increasing move toward prosecuting specific individuals who obtained or procured the drugs used by a decedent proximate to the time of their death for drug delivery resulting in death, also known as drug delivery homicide, and “but-for” causation. In addition, under federal law, in large-scale drug trafficking cases, drug distributors, or purveyors, including prescribing physicians in “pill mill” cases, members of transnational organized crime groups and illegal drug distribution syndicates, are liable for significant enhanced penalties including 20-year mandatory minimum sentences if deaths resulted as a direct result of their involvement in the distribution of their product.

In 2014, the United States Supreme Court handed down *Burrage v. United States*, which constitutes prevailing authority on the standard of causation required for imposition of the federal sentencing enhancement.<sup>1</sup> The court focused on the specific text found in 21 U.S.C. § 841(b), the federal law requiring heightened sentences for drug sales causing death or serious bodily injury. In a unanimous decision, the Court ruled that a defendant cannot be held liable under the penalty enhancement provision of the Federal Controlled Substances Act unless a “but-for” cause of death or injury is established due to the distribution of a particular drug or drugs. The Court also held that the federal sentencing enhancement applies when it is proven beyond a reasonable doubt that the drug or drugs distributed by the defendant are “an independently sufficient cause of the victim's death or serious bodily injury.” The opinion states that the Government does not have to prove that the drug/drugs were the only cause of the decedent's death; but it must have been “the straw that broke the camel's back.”

The *Burrage* opinion has engendered differential interpretations in lower federal and state courts, which has resulted in corresponding ambiguities and incertitude among the scientific community called to testify in such cases. Among the challenges of presenting this evidence is the fact that many of these cases involve deaths of individuals who have been using multiple drugs that may or may not have come from the same supplier, and the timing of ingestion of the specific substances, the decedent's potential tolerance to the effects of the drug, and other factors are unknown.

This workshop will review the underlying Federal and corresponding state statutes that address these issues and how cases are being charged and prosecuted. The complex nature of certification of drug-related deaths, especially in the context of multiple mixed drugs being detected in the toxicological analysis, will be reviewed. Some exemplar case scenarios, and identifying key corroborating elements of physical evidence scene investigation, medical history, or eyewitness testimony that may weaken or strengthen the introduction of expert testimony will also be reviewed.

The expert panel will provide perspectives on the nuances of the terms “independently sufficient cause,” “resulting from,” “but-for causation,” and the interpretation of the “straw the broke the camel's back” analogy from both a legal and an applied science perspective. This presentation will lead the attendees to appreciate that the importance of the evidence that supports or undermines confidence in the expression of these scientific opinions in the context of charging, prosecuting, defending, testifying, and achieving just sentences in cases of this type.

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#### Drug Delivery Homicide, But-For Drug Death Causation, Drug Delivery Resulting in Death



### W2 Statistical Learning Algorithms for Forensic Scientists

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**Learning Overview:** The goals of this workshop are to: (1) introduce attendees to the basics of supervised learning algorithms in the context of forensic applications, including firearms and footwear examination and trace evidence, while placing emphasis on classification trees, random forests, and, time permitting, neural networks; (2) introduce the concept of a similarity score to quantify the similarity between two items; (3) show how learning algorithms can be trained to classify objects into pre-determined classes; (4) discuss limitations of Machine Learning (ML) algorithms and introduce methods for assessing their performance; and (5) discuss the concept of a Score-based Likelihood Ratio (SLR): computation, advantages, and limitations.

**Impact on the Forensic Science Community:** The use of learning algorithms will increase as measurement of features in various types of evidence improve. This is particularly true in the case of pattern evidence. Forensic scientists will greatly benefit from understanding the basic ideas that underpin statistical learning since these types of methods have already been proposed for firearms examination, fingerprints, glass comparison, and shoe print evidence.<sup>1,2</sup> Most quantitative training for forensic scientists emphasize classical statistical ideas, so a workshop in which forensic practitioners are exposed to learning algorithms is novel and timely.

When a task consists of deciding whether two items are similar enough to suggest that they could have a common source, an alternative approach is to use statistical or machine learning. Machine learning is the term used to refer to a family of statistical methods and computer algorithms that find patterns in data and has been around for decades.<sup>3</sup> There are many different types of algorithms, but a basic taxonomy is to distinguish between *supervised learning* algorithms and *unsupervised learning* algorithms.<sup>4</sup> The focus of this presentation is on supervised learning methods.

Supervised algorithms rely on training data, for which ground truth is known, and on test data, on which the performance of the algorithm can be tested. In a simple example, several bullets are fired from a large number of guns. To train an algorithm to recognize whether a pair of bullets was fired from the same or from a different gun, one might begin by creating all possible pairs of bullets, and compute, for example, the difference in the average striation depth for each pair. This difference is a feature, and perhaps it takes on low values when two bullets were fired from the same gun and high values otherwise. Presented with the value of the feature for pairs of bullets known to have been fired from the same or from different guns, the algorithm then “learns” that same-gun bullets tend to exhibit values of the feature in a certain range that is different for different-gun bullets. With this knowledge, the algorithm can then classify other pairs of bullets for which it does not know in advance whether the bullets were shot from the same or from a different gun.<sup>5</sup>

In real applications, the number of features can be very large, and the number of classes can also be large. In classification examples, the response variable—or class—is discrete, but algorithms can also be used when the response is continuous; in this case, the problem is to predict the value of a variable given information on a large number of features.

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#### Machine Learning, Algorithms, Similarity Score





### **W3 Recent Advances in Understanding Fire Pattern Production**

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**CANCELED**



### W4 The Impact of the 2018 Farm Bill on the Forensic Analysis of Cannabis

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**Learning Overview:** After attending this presentation, attendees will understand the legal and forensic landscape of marihuana/hemp analysis and also understand a validated analytical approach that meets these new requirements.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a framework for meeting the forensic requirements of the 2018 Farm Bill.

In December 2018, the President of the United States signed into law the Agriculture Improvement Act of 2018 (Farm Bill). This law introduced two significant changes to our drug laws. First, the term “hemp” was defined as, “the plant *Cannabis sativa* L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9 Tetrahydrocannabinol (THC) concentration of not more than 0.3 percent on a dry weight basis.” Second, the law excluded the term hemp from the definition of marihuana in the Controlled Substances Act. In response to the changes at the federal level, states have enacted their own varied legislation to allow for hemp cultivation and processing and the sale of hemp products. This also led to additional analytical challenges for the states.

This workshop will provide an overview of changes in the law, describe an analytical approach to providing reliable results that conform to the new legal requirements, and provide an overview of the work conducted to validate the methods incorporated into the analytical approach. State laboratory managers will describe their varying approaches to meeting these new challenges from a state that had legalized marijuana prior to changes in federal law and a state that had not.

Analytical testing requirements of suspected cannabis submissions to seized-drug laboratories have changed. Formerly used testing protocols—analytical schemes—included tests such as macro/microscopical analysis, the Duquenois-Levine color test, and separation analyses like thin-layer- Gas Chromatography/Flame Ionization Detector (GC/FID) and Gas Chromatography/Mass Spectrometry (GC/MS). These testing procedures allowed for the identification of cannabis, but they are no longer sufficient to distinguish between marihuana and hemp because of the new requirement to quantitatively analyze the sample and assess the THC content (the quantitative property that separates hemp from marijuana).

To address the new legal requirements, Drug Enforcement Administration (DEA) scientists developed a new testing protocol. The new analytical scheme includes macro/microscopical analysis (to identify cannabis plant material), a cannabis typification color test (to assess the ratio of THC to Cannabidiol (CBD)), and GC/MS (to identify THC and assess THC concentration at the 1% level). This workshop will guide attendees through the background and design of this new testing protocol, including the method development and validation and implementation processes, as well as revised reporting language.

Additionally, scientists from the Virginia Department of Forensic Science, where marijuana has not been legalized, collaborated with the Drug Enforcement Administration (DEA) in the development and validation phases of the project. Validation studies were performed on the 4-aminophenol color test with a variety of concentration combinations of CBD reference materials and their acids, as well as non-controlled substances. The DEA 1% assessment method was further evaluated to include GC/MS instruments with a second chromatographic column and FID. This allows comparisons of peak height and peak area data from both detectors.

In Colorado, recreational marijuana and hemp were allowed by legislation passed in 2012, known as Amendment 64. As scientists considered the draft of the 2018 Farm Bill, the Colorado Bureau of Investigation selected an instrument and developed and validated a method to quantify THC in plant materials. This workshop will describe the process and the many lessons learned in the evolving area of forensic analysis.

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### Marijuana, Hemp, Forensic Analysis

### W5 The Psychopathology of Homicide

Alan R. Felthous, MD\*, Saint Louis University School of Medicine, St. Louis, MO 63104-1027; Felice F. Carabellese, MD\*, University of Bari, Bari 70124, ITALY; Giancarlo Di Vella, MD, PhD\*, University of Torino, Department Public Health Sciences, Torino 10126, ITALY; Carlo P. Campobasso, MD, PhD\*, University of Campania, Napoli 80138, ITALY; Gabriele Mandarelli, MD, PhD\*, University of Rome "Sapienza," Rome 00100, ITALY; Ilaria Rossetto, PhD\*, Residences for Execution Safety Measures, Castiglione delle Stiviere 46043, ITALY; Silvia Trotta\*, Institute of Legal Medicine, Bari 70124, ITALY

**Learning Overview:** After attending this presentation, attendees will better understand the relationship between psychopathology and the phenomenon of homicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how the identification of the features of psychopathology of homicide can inform treatment planning.

**Introduction:** Homicide is the most serious form of antisocial behavior. Despite its personal, social, and economic impact, homicide is among the least studied comprehensible forms of antisocial and violent behavior. The relationship between major psychiatric disorders and homicides has been much debated in the scientific literature.<sup>1-4</sup> Although it is known that most extreme violent acts are committed by subjects without any history of psychiatric pathology, some research suggests there is a correlation between schizophrenia spectrum disorders, severe personality disorders, and homicide.<sup>2,4</sup>

The homicides associated with a psychotic dimension present specific characteristics.<sup>3</sup> The relationship between the murderous ability to plan an antisocial act does not necessarily exclude a possible diagnosis of schizophrenia. Even more controversial is the relationship between the psychopathic dimension and homicide. Psychopathic homicides are usually goal-oriented. A non-psychopathic murderer usually commits a crime with a different type of violence, often as a result of frustration. Moreover, some gender differences can also be observed in the homicide phenomenon, whether the murder is committed by an offender affected by a psychotic disorder or is committed by a man or a woman with a psychopathic personality profile. Besides the diagnosis, it is important to point out that usually women express this antisocial behavior in a different way compared to men.<sup>3</sup>

In homicide, suspected homicide, and other suspicious or obscure cases, the role of an experienced forensic pathologist at the scene of death is generally to assess the environment, the local circumstances, and the position and the condition of the body.<sup>5-7</sup> Homicide grows out of a transaction between individuals, which is a product of the individuals and their relationship. Behaviors can thus be seen to follow a definite pattern that can be related to underlying psychological principles, which closely relate to how an offender interacts with the victim at the crime scene.<sup>8-10</sup>

Homicide can occur together with suicide in the same episode. An earlier classification of combined homicide-suicide, which considered the perpetrator's psychopathology and relationship with the victim(s) will be updated to incorporate recent studies. Individual acts of homicide-suicide can be understood by considering three contributing factors: (1) ego weaknesses or psychopathology of the perpetrator, (2) stressors, and (3) the psychological vector directing this specific act. Studied attention will be given to female homicide-suicide perpetrators and gender differences. Within this context will be considered homicide-suicide terrorists and female bombers in particular.

In the light of these premises, it can be understood how the forensic expert evaluation of the criminal responsibility of murder defendants presents specific difficulties, as well as having a significant impact in the criminal trial. Crime scene investigation requires a multidisciplinary approach by an expert team in order to shed light on difficult cases, such as murders staged as suicides or accidents.

This workshop's speakers will debate from different point of views the scenarios outlined above. The psychopathology of men and women who kill; psychopathology of men and women of criminal organizations, such as the Sicilian mafia; combined homicide suicide; the Not Guilty by Reason of Insanity (NGRI) killers—a national sample; forensic psychiatric evaluation of defendants in homicide cases—literature analysis and forensic cases; and treatment outcomes following a conviction of killing. Several general pathologists will speak on topics that pertain to the killer's mental state from the perspective of forensic pathology and investigation of homicide. The criminological and psychiatric data from a multicenter national Italian sample of forensic psychiatric patients will be also discussed.

**Conclusions:** The main purpose of this workshop is to highlight the pathological aspects of homicide: attendees will acquire information about the psychopathology of homicide from the different presentations, in which there will be an opportunity to describe the history of the disease, the treatment, the outcomes, and the criminal history of individuals who committed the crime of murder without mental responsibility because of their psychiatric illness. The speakers with different professional experiences will include in their presentations original data from recent not-yet-published investigations.

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#### Homicide, Psychopathology, Suicide

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### W6 High-Impact Practices in Forensic Science Education

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**Learning Overview:** After attending this presentation, attendees will learn how High-Impact Practices (HIPs) can be used in forensic science education and discover how to adapt their courses using common programing for students by: (1) identifying and describing a variety of HIPs; (2) recognizing useful HIPs dependent on the environment; and (3) discovering innovative HIPs that could be incorporated into their classroom.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring a variety of HIPs and gaining insight into a broader scope of common methods useful in forensic science teaching. By discussing multiple types of HIP techniques and the application of these practices in forensic science education, attendees will be able to incorporate tools into their forensic science classrooms and programs.

HIPs are defined as active learning practices that promote deep learning through student engagement. Based on this definition, there are ten different learning experiences identified as HIPs: (1) first year seminar and experiences; (2) common intellectual experiences; (3) learning communities; (4) writing intensive courses; (5) collaborative assignments and projects; (6) undergraduate research; (7) diversity/global learning; (8) service learning or community-based learning; (9) internships; and (10) capstones courses / projects. These increase student engagement and success throughout their college career. Students should participate in at least two experiences throughout college, but it is highly recommended they participate in one experience per year.<sup>1</sup> Due to the importance of these experiences in students' college careers, these are also required for forensic science programs accredited through Forensic Science Education Programs Accreditation Commission (FEPAC). This workshop explores seven ways these can be incorporated in forensic science curriculum.

Over the years, there have been issues on student success, retention rate and attrition rate. A detailed insight on the impact of First-Year Seminar Experience (FYSE) on the forensic science program at Albany State University that resulted in increased retention and graduation rate will be provided. Data will be shown on the impact of FYSE and the way forward to repositioning of the program to enable greater learning experiences for students.

Living-Learning Communities (LLC) promote collaborative learning experiences by grouping students of a similar interest or major within the same area of a residence hall. LLCs focus on students' curricular experiences to create purposeful connections between the academic and social environments of college life. The development and organization of the LLC at the University of Central Oklahoma and examples of student growth and transformation will be discussed.

The "Forensic Science Seminar at Sea" and "Field Study in Forensic Science" are two programs implemented by the School of Criminal Justice, Forensic Science, and Security at the University of Southern Mississippi. These programs provide student-centered instruction by working with forensic scientists across multiple agencies. Topics will include program planning, curriculum development, risk management, benefits, and meeting guidelines for academic accreditation.

The case practicum model provides students with information to independently perform evidence assessment and laboratory analysis in a realistic forensic case setting. Active involvement from forensic science practitioners, an extensive peer review process, and completion of a forensic proficiency test contribute to this hands-on course. An overview of Boston University's practicum course, including insight into planning, delivery, and assessment, will be shared.

Project-based learning promotes critical thinking and problem-solving skills. Coupling this concept with casework and scholarly research bridges the real expectations and outcomes in forensic investigations and research expected in academia. Using current missing person cases, students complete an experiential learning project and their work has the potential to impact a real case.

A series of semester-long projects are used to create problem-solving methods used in a forensic chemistry course. The project supplements lecture and labs in crime scene and laboratory forensics with separate tracks related to bloodstain pattern interpretation, gamification of a forensic chemistry lab, and developing contemplative methods to enhance observational skills. The lectures, projects, and assignments that accompany these three tracks will be discussed.

The University of Central Oklahoma's (UCO's) Student Transformative Learning Record (STLR) is a non-proprietary model with a proven track record for increasing retention, student academic achievement, and workforce readiness. An overview of the STLR process, how it is assessed, and examples of transformational learning experiences within the UCO Forensic Science Institute will be shared.

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#### Education, High-Impact Practices, Student Experiences

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### W7 New Advances in Forensic Human Identification. Issues and Approaches

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**Learning Overview:** After attending this presentation, attendees will have a heightened understanding of: (1) the factors affecting human identification; and (2) how the application of new, innovative, and cross-disciplinary techniques have the potential to overcome these issues, which will be detailed through case examples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through the presentation of multidisciplinary approaches toward human identification, pointing out the novel techniques suitable for this purpose, encouraging collaboration and innovation among different forensic specialties to document and integrate several lines of evidence.

Human identification always has been a complex task; the degree of complexity, however, is highly dependent on the context in which death occurred. Phenomena such as the human mass migration and displacement witnessed in the past years add challenge, such as an increased difficulty in tracing an individual's geographical origin, thus complicating antemortem data and reference sample collection.

The traditional identification approaches of forensic anthropology and odontology, as well as their advantages and limitations, will be covered before delving into the introduction of new forensic procedures. Additionally, challenging cases of human identification will be presented, and step-by-step approaches on how to tackle them from different disciplines will be delivered.

Time frame and geographical origin of the remains are crucial data to achieve a positive identification of the unknown. New advances in microbiota characterization can provide insights about the time-since-death; additionally, the analysis of radiocarbon can offer important information regarding when the individual was born and died. The estimation of a fingerprint's age can provide valuable information that can help relate an individual temporally in a certain location.

Geographical origin can be established by isotope analysis, also providing information about the diet of the deceased.

New trends in forensic genetics point out the use of Small Nucleotide Polymorphisms (SNPs) to determine eye and hair color and ancestry, leading to a facial approximation based on genetic traits. In the past years, 2D and 3D imaging techniques have been developed and have been very useful in forensic science, especially for comparison of postmortem and antemortem images, and several of these techniques will be demonstrated.

This workshop will further cover the topic of appropriate documentation and temporary disposition of unidentified remains in order to maximize the potential for future identification.

All these new and innovative methods will help forensic scientists create the biological profile of human remains and achieve a positive identification.

This workshop will demonstrate how the awareness of the problems faced in human identification and thinking out-of-the box and applying and integrating new techniques with traditional procedures are essential to facilitate the correct identification of the victims. This is particularly important in mass disaster scenarios, where the large number of deceased pose challenges to this task as human remains are often incomplete, commingled, and sometimes only fragmentary remains are present. Such complexities require comprehensive approaches and new tools that contribute to obtaining different lines of evidence to positively identify the deceased. Thus, this workshop will provide a step toward finding solutions to these complications by improving the knowledge on the most state-of-the-art procedures available to forensic identification.

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#### Human Identification, Multidisciplinary Approach, State-of-the-Art Forensic Technique



### W8 Death Investigations in the Military: Case Studies From Around the World

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**Learning Overview:** After attending this presentation, attendees will understand: (1) processing a variety of death scenes; (2) forensic capabilities of military investigators; (3) investigative considerations in foreign countries; (4) coordination with international agencies; (5) domestic violence impact on child death; (6) staged suicide; (7) scene reconstruction; and (8) laboratory capabilities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through detailed accounts of a variety of death investigations as described by Army Special Agents who participated in the cases. The investigations represent various circumstances and illustrate the worldwide capabilities and responsibilities of military investigative agencies, along with legal and laboratory support.

In June of 1956, a soldier assigned to Fort Riley, KS, failed to show up to his assigned place of duty and was declared Absent Without Leave (AWOL). Fifty years later, a family in New Brunswick, Canada, came forward with the information that their grandfather was the missing soldier from Fort Riley. Army Criminal Investigation Command (CID) working with numerous agencies verified (through DNA) the grandfather in Canada was the missing soldier from 1956. The investigation helped bring some answers and closure to both families.

In 2018 an 11-year-old committed suicide at an Army installation. Investigation into the death of the child found numerous law enforcement interactions with the family at different Army installations, including child abuse involving the victim and his siblings. Domestic violence by the stepfather and biological mother included a firearm-related incident. The firearm was returned by law enforcement and was used in the suicide.

During the draining of a lake, a vehicle antenna appeared on the surface. Divers identified a submerged car and found a foot stuck under the driver's headrest. Identifying the victim, recovering data from a cell phone, and following leads more than five years old led to a suspect. The suspect ultimately confessed to strangling the victim, driving the car onto post, and submerging the car in the lake.

In March 1991 a soldier's wife was found dead with a single gunshot wound to the head, pistol in hand. Several suicide notes and a history of depression and marital problems led to a closed suicide. Later information developed, including a possible earlier attempted murder by arson. The soldier was tried for murder and attempted murder.

A case study examines a unique multiple gunshot wound suicide of a 36-year-old, Caucasian male Army service member who shot himself nine times with a Walther® P22 pistol (.22LR). Entrance wounds included the head, neck, and chest.

A soldier used a network of friends to help harass and stalk his estranged soldier wife. After seeing her with male friends, the subject and an accomplice broke into one of the other the men's residence and killed two of them.

On Christmas day, 2005, a United States soldier was attacked by a translator in Mahmoudiya, Iraq. The translator opened fire, striking the soldier, who luckily survived the attack; he returned fire, striking and killing the translator.

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### Homicide Investigation, Forensics, Suicide



### W9 Interpersonal Violence and Elder Abuse: Maltreatment That Crosses All Borders

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**Learning Overview:** After attending this presentation, attendees will be able to identify multiple forms of abuse and mistreatment, recognize the impact of abuse across the lifespan, and specify key points for the prosecution of abusers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by broadening the education of those working with victims of violence. This presentation will enhance not only the ability to intervene in maltreatment but to prosecute the abuser.

Interpersonal violence and maltreatment are recognized criminal and social problems both in the United States and internationally. Most societies considered child abuse and interpersonal violence, once called “domestic violence”, private family matters. As more research became available, intervention increased, with cases of both child and adult abuse moving into law enforcement and the criminal court system. The actual study of elder abuse is relatively recent in the United States. Mentions of elder mistreatment date back to the mid-1970s, but research and resources were years in coming. Similar to child abuse and domestic violence, harm to an older relative was considered a family matter and stayed behind closed doors. Much of early theory was based on patterns of family violence that had been studied in child abuse and the battered wife syndrome. With the growing understanding of the nature and extent of elder mistreatment came the awareness of the lack of forensic and medical markers that could be useful in the identification of abuse. There was no research that could assist investigators in establishing lacerations, bruising, fractures, or decubiti as elements of abuse or neglect. The aging patient frequently has physiological characteristics or medical conditions, which can mask or mimic markers of neglect and abuse, making it even more difficult to detect or evaluate. Unless an actual crime could be proven, such as homicide, charges were rarely filed against a suspected abuser. Arrest and prosecution for financial abuse or neglect was unheard of. Testimony in both young and older patients could be difficult to obtain.

Multiple aspects of abuse have been examined in the research, including measures for detection, assessment, and documentation, and yet crimes of abuse continue to be difficult to document and prosecute. In this workshop, attendees will learn the challenges of detection and intervention of abuse and the tools that lead to successful prosecution in criminal cases of abuse. The impact of trauma on the victim’s health will be discussed, with specific examples of the impact of strangulation, neglect, and self-neglect. Examples from the literature on child abuse will be used as illustrations. Age-related changes and co-morbidities will be presented, with emphasis on differentiating abuse from other conditions. The intersection of elder abuse and interpersonal violence will be explored. Investigating abuse and neglect in health care facilities is presented, with an intensive case review of elder sexual abuse. The response to the elderly in disasters will be described, with a panel review at the end of both the morning and afternoon sessions.

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#### Interpersonal Violence, Abuse, Maltreatment



### W10 The Investigation and Analysis of Health Care Serial Killers

*Laura M. Labay, PhD\**, NMS Labs, Willow Grove, PA 19090; *Donna M. Papsun, MS*, NMS Labs, Willow Grove, PA 19090-2910; *Michael M. Baden, MD*, New York, NY 10019 *Beatrice Yorker, JD\**, California State University, Los Angeles, Los Angeles, CA 91103; *Steven M. Marcus, MD\**, Montville, NJ 07045; *Tim Braun\**, National Center for Missing & Exploited Children, Alexandria, VA 22314; *Paul Uribe, MD\**, Armed Forces Medical Examiner System, Dover Air Force Base, DE 19902; *Kevin M. Legg, PhD\**, Center for Forensic Science Research and Education, Willow Grove, PA 19090; *Vincent Marks, DM\**, University of Surrey, Guilford, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will: (1) state the different motivations of health care serial killers; (2) understand some of the challenges associated with analytical testing and result interpretation; (3) list the agents that have been used in medical killings and describe their mechanism of action; and (4) understand how to best prepare for case presentation in a legal proceeding.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a multidisciplinary approach to understanding, identifying, and investigating health care serial killers.

A health care serial killer, sometimes referred to as an Angel of Death or an Angel of Mercy, is a unique type of criminal offender who, while employed as a medical provider, intentionally ends the life of a patient. Infamous examples include: physician Michael Swango, who is estimated to have killed more than 60 people; LVN Genevieve Jones, who murdered infants and children; and physician Harold Shipman, who killed hundreds of patients. The motivations are varied, but include ending the suffering of a patient who likely will not recover, minimizing the emotional toll on relatives and loved ones, achieving recognition from their colleagues by accomplishing life-saving resuscitation measures, and exerting dominance over a helpless or weaker individual.

These cases are challenging to recognize as they typically occur over an extended period of time, involve different employers, and include patients who are severely ill and/or have a terminal diagnosis. The latter situation means that because a reasonable competent cause of death already exists, the death may not be heavily scrutinized. Furthermore, analytical evidence or proof may be lacking since hard-to-detect substances, such as epinephrine, succinylcholine, insulin, and heparin, may be used. Instead, identification and in-depth investigations tend to occur when a correlation between a care provider's presence and a cluster of deaths is observed. A high-profile prime example is the Cullen case. Cullen was a nurse who was able to move from facility to facility in New Jersey and Pennsylvania in spite of his questionable employment record and even though suspicions were raised by coworkers that he was harming patients. He eventually confessed to killing more than 40 patients over the course of 16 years.

Medical and forensic science practitioners must be well-versed in detecting these deaths and, when required, be equipped to perform a thorough and lengthy investigation. Due to the complexity of these cases, most will require a multidisciplinary approach and include input from legal investigators, toxicologists, and forensic pathologists. Another facet is the prevention of these deaths. For example, what systems are in place at care facilities to monitor the dispensing and use of medications, and are there any mechanisms or safeguards from a psychiatric standpoint that allow for the profiling and identification of at-risk care providers? Greater insight learned from previous cases and more understanding about the perpetrators may aid in limiting future deaths and helping direct investigations.

#### Medical Killers, Health Care Serial Killers, Angels of Death





## Workshops—2020

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### **W11 The Native American Graves Protection and Repatriation Act (NAGPRA) and the Medicolegal System: Legal, Practical, and Ethical Considerations for Practitioners**

*Megan K. Kleeschulte, MA\**, University of Tennessee, Knoxville; *Amy Mundorff, PhD*, University of Tennessee, Knoxville; *Ellen Lofaro, PhD*, University of Tennessee, Knoxville; *Bruce E. Anderson, PhD*, Pima County Office of the Medical Examiner, Tucson, AZ

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**CANCELED**



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### W12 KidStats: Improving the Subadult Biological Profile

*Kyra E. Stull, PhD\**, University of Nevada, Reno, Reno, NV 89557; *Heather M. Garvin, PhD\**, Des Moines University, Des Moines, IA 50312-4198; *Alexandra R. Klales, PhD\**, Washburn University, Topeka, KS 66621; *Louise K. Corron, PhD\**, University of Nevada, Reno, Reno, NV 89557; *Michael H. Price, PhD\**, Santa Fe Institute, Santa Fe, NM 87501

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**Learning Overview:** After attending this workshop, attendees will be trained on the most up-to-date techniques to estimate subadult age and sex and on KidStats, a freely available web-based graphical user interface.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with applied experience in collecting subadult data from 3D printed remains of individuals between birth and 15 years and the application of these data in a statistical framework.

A lack of modern subadult samples has greatly limited the development and validation of subadult biological profile techniques, as well as practitioners' experiences working with subadult remains. The computer tomography sample of more than 1,100 individuals aged between birth and 20 years, collected from the University of New Mexico Office of the Medical Investigator and from the Office of the Chief Medical Examiner in Baltimore MD, enabled this study to collect more than 200 variables (i.e., age and sex indicators) from most individuals, resulting in an extraordinary amount of knowledge regarding skeletal and dental growth and development and variation within a large, modern sample of North American children. This workshop will take the attendees through the subadult skeletal research experience, from research design challenges to the results obtained, as well as a discussion on how the project results vary from previous publications.

Although subadult age estimation has been historically considered a reliable practice, current age estimations continue to face limitations. For example, methods tend to focus on only a single variable, such as dental development. One of the main methods that will be discussed is the mixed cumulative probit algorithm that was designed specifically for dealing with continuous (diaphyseal dimensions) in conjunction with ordinal age indicators (epiphyseal fusion and dental formation) to predict a continuous outcome variable (age). Previously in subadult age estimation techniques, the age estimation methodology has been limited to a single variable and a single indicator without any statistical way to combine different age estimations. This model enables individuals to enter any combination of data for epiphyseal fusion, diaphyseal dimensions, appearance of ossification centers, or dental formation and yields one age estimate with 95% confidence intervals, thus meeting the best practices of our field. Not only is this novel algorithm applicable in KidStats, it is also freely available in an R package that has been released. While the main focus of the workshop is on age estimation and KidStats, other topics (or research) will be presented, including the utility of the subadult pelvis for sex estimation, subadult body mass estimation, and subadult stature estimation.

Overall, this workshop seeks to provide attendees with experience working with subadult remains, familiarize them with modern techniques in estimating subadult biological profile parameters, and ensure they understand both the capabilities and challenges in performing such analyses. Three-dimensional (3D) prints of subadult remains will be used to guide attendees through the newly proposed age and sex methodologies. Because subadult skeletal samples are rarely available, the 3D printed material provides a unique opportunity for attendees to see how age and sex indicators vary throughout ontogeny.

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### Forensic Anthropology, Biological Profile, Juvenile



### W13 A Primer on Multidisciplinary Investigations of Pediatric Injuries and Deaths

*J.C.U. Downs, MD\**, *forensX, LLC, Savannah, GA 31406*; *Joanna L. Collins, MFS, LINUS Investigations & Consulting, San Antonio, TX 78258*; *Bonnie Armstrong, BS\**, *The Shaken Baby Alliance, Boyd, TX 76023*; *Robert N. Parrish, JD\**, *Salt Lake County District Attorney's Office, Salt Lake City, UT 84111*

**Learning Overview:** Attendees will be able to demonstrate the ability to analyze and evaluate available data in a pediatric death or injury case to formulate a viable hypothesis regarding the actual mechanism of the pathology in question. After attending this presentation, attendees will be better able to differentiate and implement effective investigative techniques from lesser alternatives.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by exposing practitioners to an effective multidisciplinary team approach to prospectively work through investigations as they progress to a conclusion.

Pediatric cases are some of the most difficult cases for investigators to work, particularly those involving the injury and/or death of a young child. Multiple intersecting stressors can impact the investigation, as well as the investigator. Medical findings may be equivocal or complicated. Subtle and/or minimal findings pose diagnostic challenges, while physical injuries, especially to vulnerable victims, can be emotionally challenging. Existing medical conditions can add a layer of confusion while excluding possible mimics, or other conditions may prove labor intensive. In addition, parents, families, and communities want answers. Thorough investigations require enough time to ascertain the truth—whatever that might be. All these, and other, aspects tax the limited resources available to the investigator. In cases where the justice system becomes involved, all the myriad elements receive further intense scrutiny.

Multidisciplinary teams have proven popular in providing a retroactive analysis in dealing with such cases, due to their significant successes. Key elements in such cases involve the clinical medical team, forensic pathologist, social workers, advocacy center, law enforcement, legal community, and others. These teams are obviously targeted at deceased individuals; however, there exists no reason why the same concept cannot be applied with equal effort to living victims. Likewise, if applied at the inception of a case moving forward, as the investigation is ongoing, such multidisciplinary collaboration can achieve excellent investigative conclusions.

This workshop will condense a two-day workshop into a one-day overview of how this concept can be applied in practice. All these cases begin with the medical recognition of suspicious child deaths and injuries and, as such, a review of common findings may help in isolating elements that make some deaths “suspicious” or worthy of further scrutiny. Once concerns are raised in a case, the typical investigation escalates from routine to a more intensive and focused search for answers. A large part of attempting to ascertain the truth of events will arise from informal and formal interviews of persons with knowledge about case particulars. A critical element to better understand events is the development of a timeline allowing visualization of potential inclusionary and exclusionary information. Such a process is very similar to the singularly most important diagnostic element in medicine—establishing a patient history based on open-ended, direct questioning of person(s) with direct knowledge about the patient’s condition.

Using this investigative model, the technique will then be demonstrated in one of the more challenging and contentious areas of forensic medical practice: pediatric head injuries. Recognizing that there are differing points of view about how such trauma occurs and presents is critical to understand an individual case. Reflex application of dogma can hinder impartial case analysis. As such, an objective overview of present medical knowledge will be mixed with investigative techniques and timeline development to arrive at an investigative endpoint, regardless of whether it tends to inculcate, exculpate, or provide no definite answer.

This workshop is intended to enhance attendees’ knowledge of excellent techniques for the multidisciplinary investigation of pediatric injury and/or death, positioning them to better implement objective analysis and appraisal of case data to formulate an overall evaluation for potential subsequent action(s), if needed. Such a strategy would improve patient outcomes by enhancing the final investigative conclusions.

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#### Multidisciplinary, Pediatric, Trauma



### W14 Mass Disasters and Disaster Victim Identification (DVI)

*John A. Williams, PhD\**, Western Carolina University, Cullowhee, NC 28723; *Victor W. Weedn, MD, JD\**, The George Washington University, Washington, DC 20007; *Jason M. Wiersema, PhD\**, Harris County Institute of Forensic Science, Houston, TX 77054; *Anthony B. Falsetti, PhD\**, College of Science, Forensic Science Pro, Fairfax, VA 22030; *Kenneth W. Aschheim, DDS\**, New York, NY 10065; *Bryan Johnson, MSFS\**, Federal Bureau of Investigation, Quantico, VA 22135; *Taylor M. Dickerson III, MSFS\**, SNA International, Armed Forces DNA Identification Laboratory, Dover Air Force Base, DE 19912; *Jason H. Byrd, PhD\**, University of Florida, Gainesville, FL 32608; *Cynthia Gavin, PhD\**, Parkville, MD 21234

**Learning Overview:** After attending this presentation, attendees will be able to: (1) understand the historical background of DVI, (2) recognize the need for standards and best practices, (3) understand the integrated contributions of the various forensic sciences to DVI, and (4) understand the critical relationship of the Victim Information Center to the identification process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with an understanding of the current state of mass fatality victim identification as it exists in the United States. Presentations will encompass the breadth of the forensic sciences, including ethical considerations and the role of the Victim Information Center in mass fatality identifications.

DVI is the comprehensive process of human identification as applied to mass fatality events. Although by definition a mass fatality event is any situation that overwhelms local resources, we generally think of a situation in which identification is hampered by the event and recovery process. Human remains that have been badly traumatized, heavily decomposed, or recovered outside of their normal context are examples that fit this description.

In 2014, responding to the 2009 NAS Report, the United States Department of Commerce's National Institute of Standards and Technology (NIST) established the Organization of Scientific Area Committees (OSAC) to assist the development of standards in the forensic sciences. The OSAC includes a DVI subcommittee that has promulgated recommended standards. In 2016, the American Academy of Forensic Sciences' American Standards Board (ASB) was established as a Standards Development Organization (SDO). The ASB includes a DVI Consensus Body to produce American National Standards for DVI. Using the OSAC DVI recommendations, the ASB has published several DVI standards and best practices.

While DVI may take place locally, it is by its nature a governmental process (i.e., based in the office of a coroner or medical examiner). At the United States Federal level 15, Emergency Support Functions (ESF) outline how the public and private sectors respond to national emergencies. Buried in the scope of ESF#8 is the role of the Public Health Service in disaster response, specifically mass fatality management and DVI. Here is where standards or best practices of DVI would prove highly beneficial in providing guidance to the varied agencies that may be involved in a mass fatality event.

Human identification, and by association DVI, has changed over the past century. In the not-too-distant past, visual identification was the norm. The advent of fingerprints and later odontology led to dramatic improvements in identification, especially after decomposition or traumatic injury has taken place. Today, these so-called traditional methods, along with anthropology, still dominate the field of human identification. Continued improvements in DNA technology point to the direction that the future of DVI will be taking. Time and cost are deciding factors in DVI; expedient and cost-effective methods will not be falling by the wayside any time soon.

An often neglected, but immeasurably critical, aspect of DVI is the gathering of antemortem information concerning the victims. Identification would be impossible, or nearly so, without the Victim Information Center. Interviews of family members and others acquainted with the victim's identifying features, as well as medical and dental records, are collected and compared against the biological profile of the victim's remains.

DVI therefore is the concerted and combined efforts of dedicated scientists, specialists, and governmental and private agencies. It is practiced under far less-than-perfect conditions under circumstances that are often politically and culturally charged.

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#### Identification, Disaster, Victim



### W15 Dementia Workup for Forensic Pathologists Following a Condensed National Institute on Aging-Alzheimer's Association (NIA-AA) Protocol

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**Learning Overview:** After attending this presentations, attendees will: (1) become familiar with the diagnostic workup and grading schemes for common classes of dementia; (2) be able to accurately identify and sample appropriate regions of the brain according to the Condensed Protocol for dementia workup; and (3) understand the benefits and limitations of the Condensed and NIA-AA Protocols for dementia workup.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a method that reduces the cost of histopathologic dementia workup while maintaining diagnostic performance.

Alzheimer's Disease (AD) is the fifth most common cause of death for Americans ages 65 and older.<sup>1</sup> In the United States, the number of people affected by AD and related dementias is projected to increase substantially, rising from an estimated 5.4 million in 2015 to 11–16 million by 2050.<sup>2</sup> Individuals who die with dementia may be susceptible to non-natural factors such as neglect, abuse, or accidental injury, and investigation into potential dementia cases will likely be increasingly common in the forensic autopsy setting. Definitive diagnosis of a specific type of dementia necessitates neuropathological examination, which if performed according to the Original NIA-AA guidelines (20 blocks and 13 special stains from key brain regions), may be cost-prohibitive in a forensic setting.<sup>3,4</sup> In response, a Condensed Protocol was developed that consolidates the sampling and staining proscribed by the Original NIA-AA Protocol into five cassettes for a single round of histochemical or immunohistochemical staining, resulting in approximately 75% lower cost (institution specific) while maintaining diagnostic performance.<sup>5,6</sup> The Condensed Protocol has been studied for use in a forensic setting and was found to be a useful tool in diagnosing a wide range of neurodegenerative diseases.<sup>6</sup> One drawback of the Condensed Protocol is decreased sensitivity for detecting Microvascular Lesions (MVL), which can be remedied by submitting additional Hematoxylin-Eosin (H&E) sections when MVL represents a strong consideration. While not intended as a replacement for the original NIA-AA guidelines, the Condensed Protocol serves to make the guidelines accessible to forensic practices, maintaining reasonable diagnostic performance for various types of dementia while conserving precious resources.

This workshop will walk attendees through specific considerations for dementia evaluation in the forensic setting. There will be an overview of the Original NIA-AA guidelines for the diagnosis of AD and related neurodegenerative disorders and the creation of the Condensed Protocol. The application and performance of the Condensed Protocol at a large urban medical examiner's office will be highlighted and compared to that observed at an academic medical center clinical autopsy service. Finally, there will be a practical demonstration of the use of the Condensed Protocol with an interactive session.

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#### Dementia, Neuropathology, Cost-Effective



### W16 Forensic Multimedia Authentication: Real-Life Challenges

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**Learning Overview:** After attending this workshop, attendees will: (1) become familiar with the latest developments in forensic video and audio authentication, enhancement, and restoration; (2) understand criteria used for media authentication; (3) understand how to conduct analysis within a forensic framework; and (4) explore the latest technologies in the generation of synthetic imagery, including deepfakes, face2face, and others.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by: (1) explaining the scientific approach in forensic media authentication, enhancement, and restoration; (2) demonstrating an authentication investigation framework; and (3) discussing tools used to create and combat multimedia forgery.

Digital multimedia authentication seeks to determine the validity of digital multimedia containers and contents by investigating their format, structure, time, frequency, and pixel and/or sample level features. This workshop will discuss the multimedia authentication process, providing the user with methods of authenticating both video and audio, including deepfakes and deepvoices. This workshop will also demonstrate the incorporation of multiple tools and techniques into unified frameworks appropriate in forensic examinations where reducing examiner bias and error is crucial.

This half-day workshop will cover video and audio authentication analyses. The goal of this workshop is to provide an overall view of conducting comprehensive examinations that rely on the results of multiple analyses to inform an ultimate finding or opinion. First covered will be a video authentication framework, focusing on camera verification/identification and image and video attack detection. This includes a quick overview of the digital video file creation chain for contextual information of the artifacts that influence the final digital media streams based upon a general description of camera sensor noises for both complementary metal-oxide-semiconductor and charge couple device type sensors. Photo Response Non-Uniformity (PRNU) are small artifacts of the sensor and can be used as a sort of fingerprint for the sensor. For video and images, it can be determined with a high likelihood that a certain image or video has been made with a specific camera. PRNU can also be used for detecting deepfakes. Splicing, copy-move, and removal artifacts are also investigated in a complex video authentication process and will be discussed and exemplified with original and manipulated videos.

The use of articulated 3D face models is a double-edged sword: it can be used to make deepfakes, but it also can be used to recover true image/video details. A theory and the method for video resolution enhancement restoration with data adaptive 3D facial models will be presented. This method starts with a generic facial 3D model, then simultaneously recovers the finer facial feature details in the video frames and also adapts the 3D model to fit the observed face in the video.

In the second section of the workshop, real-life audio challenges and solutions will be presented. The proposed audio authentication framework combines both container and content analysis to determine authenticity of the recording as well as the purported source. Audio container analysis will exploit characteristics of the multimedia file format and structure while content analysis will cover time and frequency domain techniques, including quantization level, power, direct current offset, butt splice, spectral and Multidetector Computed Tomography (MDCT) analysis.

#### Multimedia Forensics, Authentication, Deepfake



### W17 The Murder of Women: A Global Issue That Demands Action

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**Learning Overview:** After attending this presentation, attendees will understand and recognize how discrimination against women prevails throughout the world and how violence against women, a serious violation of human rights, is the most severe consequence of such discrimination. The Inter-American Court of Human Rights defined femicide as “the murder of women by gender” considering that these deaths result from structural and social attitudes, rooted in a culture of gender-based violence and discrimination. Acts of violence against women based on their status as women demand the serious and specific attention of the international community.

**Impact on the Forensic Science Community:** Attendees will become more aware of the various types of violence that are manifested in many ways. Most victims are raped and many are mutilated, tortured, or dismembered. Femicide is a complex phenomenon, ranging from the murder of women by their partners or ex-partners, to their kidnapping followed by rape and subsequent killing committed by unknown perpetrators, to ritual crimes performed by sects or other groups.

This presentation will impact the forensic science community by empowering attendees to understand that is an obligation of the European Union, the United States, and the entire world to prevent and eradicate femicide and to adopt administrative, legislative, and judicial measures to prohibit gender discrimination. States must ensure the exercise of the rights of women and pursue, by all appropriate means and without delay, policies that prevent, punish, and eradicate violence against women. States have the obligation to standardize all their protocols and manuals; to update their research criteria; and to restructure the administration of justice related to investigate disappearances, sexual violence, and murder of women. These measures have implications not only for the United States, but also for other systems of human rights protection, such as the European Court of Human Rights. After implementation of various policies addressing this crucial issue, states should be held responsible for lack of diligence in investigations, lack of access to justice for the families of the victims, and, therefore, for the impunity of the perpetrators. To sum up, states are responsible for taking the necessary steps to ensure a life free of violence for women.

The study shows that is reasonable to create scientifically based monitoring with a proper strategy together with all national and international partners.<sup>1-5</sup> Such a structure must promote the creation of comparable national databases, while also systematically involving existing international systems of data collection and their experience. The database is designed to provide easy access to comprehensive and up-to-date information on measures undertaken by governments to address all forms of violence against women; increase opportunities for the exchange of experiences in addressing violence against women; strengthen the knowledge-base for effective policy responses to prevent and address violence against women; and encourage the further collection, availability, use and dissemination of data on violence against women, as well as analysis of such data.

Data are collected using a survey based on interviews of women who turn to support centers who were asked about their experiences of physical, sexual, and psychological violence, including incidents of intimate partner violence (“domestic violence”).

The research highlighted that the collection of police data alone would not be sufficient for analyzing the cases in the detail needed for prevention and intervention. Therefore, the strategy of collecting information on all the cases known to the media and collating it with the criminological data and, insofar as is possible, data recorded by health and support systems. All data together provide the opportunity to obtain more background information on femicides, which is important for future prevention and early interventions.

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#### Violence Against Women, Murder of Women, Femicide



### W18 Injury Biomechanics: An Interdisciplinary Approach and Forensic Applications

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**Learning Overview:** After attending this workshop, attendees will comprehend the advantages of using an interdisciplinary team to address questions concerning injury biomechanics and associated trauma analysis. Attendees will be exposed to the applications of injury biomechanics across multiple scientific disciplines through presentations and hands-on experiences. Additionally, attendees will be introduced to the value of experimental testing in developing and validating forensic methods of injury analysis and the importance of scientific validity and error rates.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by providing attendees with an introduction to injury biomechanics and the applications of this research to forensic anthropology, engineering and applied sciences, and forensic pathology.

Injury biomechanics is the science that relates mechanical forces to disruption of anatomical regions of the human body. As a multidisciplinary field encompassing engineering, physiology, anatomy, and medicine, an understanding of injury biomechanics would benefit forensics, specifically in regard to analyzing injuries, the mechanisms that cause them, and recreating traumatic events.

The focus of this workshop is to introduce the principal aims of injury biomechanics research and discuss how these aims are relevant in forensic fields and methods. The utilization of a multidisciplinary team encompassing injury biomechanics, forensic anthropology, forensic pathology, and human anatomy allows for a holistic approach to investigating the relationships between injury and physical mechanical properties. By doing so, identifying mechanisms of injury will satisfy *Daubert* standards and provide quantifiable methods to support expert testimonies. Ultimately, this interdisciplinary pursuit will increase accuracy and precision in the reconstruction of traumatic events.

Presentations will begin with an introduction to injury biomechanics and the importance of an interdisciplinary team of scientists. Following the introduction, attendees will learn about the applications and limitations of forensic injury biomechanics. The development of injury criteria, injury risk assessment, and injury thresholds of the human body will then be discussed. Experimental design will next be presented in detail, addressing what goes into the design, interpretation of experiments, and the importance of quantitative data and error rates. Experimental testing methods and applications will then be addressed; specifically, the role of experimental testing in the development and validation of forensic methods. Attendees will be presented with current injury biomechanics research conducted in the Injury Biomechanics Research Center focusing on the injury mechanisms of different anatomical body regions. Each research presentation will address the following: objectives and hypotheses of the project, interdisciplinary contributions, applications of the research to various fields and professions with an emphasis on forensic applications. Attendees will be provided the opportunity to observe and discuss the soft tissue and skeletal trauma associated with each presentation and discuss interpretations. At the end of this workshop, attendees will be provided with the opportunity to discuss injury biomechanics in general and direct questions to the panel of experts who are engineers, anatomists, and forensic anthropologists.

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#### Trauma, Methods, Fracture





### **W19 Think Tank on the Leading Edge of Forensic Science, Including Robotics, Drug Intelligence, Analytical Chemistry, Technology Rules, Bayesian Approach, Legal Technology Developments, Research and Development (R&D) in a Forensic Lab, Veterinary Forensics, and Aquatic Death Investigation**

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**Learning Overview:** After attending this presentation, attendees will better understand the various new advances in forensic science that promise to have an important impact on their work.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the absolute latest new developments in robotics, drug intelligence, analytical chemistry technology rules, Bayesian approach, legal technology developments, R&D in a forensic lab, veterinary forensics, and aquatic death investigations. This workshop will help attendees be on the leading edge.

The history of forensic science dates back thousands of years. Ancient Chinese were credited with being the first to attempt to define the difference between natural death and criminal intent. By the early 1800s, the recognition of fingerprint patterns was studied, but decades would pass before that observance was applied to criminal and personal identification. Now, in today's world, new developments are being established in a single year, not decades, but the real question is how long it will take for disruptive technologies and the leading edge to obtain general acceptance in the scientific community.

Legal technology developments and technology rules are expanded on by a prominent state judge with decades of experience so that the forensic community can keep up with the quickly advancing frontiers of legal technology and innovations. Analytical chemistry, statistical revelations, and R&D in the forensic lab are improving and expanding. However, the limitations and the error rates of the algorithms utilized need to be closely monitored. Artificial intelligence and robotic advancements may help us better monitor algorithm error rates.

Aquatic death/abuse investigations are also discussed to reveal how the investigators have been revolutionizing scene investigation standards. The use of proper and specialized training, while it may take up more time initially, will provide better accuracy and understanding of the common errors repeated for decades in scene and aquatic death investigations.

With regard to investigating animal abuse, neglect and cruelty will also be discussed, including how veterinarian forensic experts are able to properly interpret evidence and ensure animal welfare.

Drug intelligence has been an important topic of interest due to the increase of distribution of synthetic novel drugs. Exploration is needed in how government agencies, law enforcement officials, forensic laboratory personnel, medical examiners/coroners, researchers, and other experts can collaborate in order to respond to increasing problems that are seen with drug abuse.

This year's think tank is a collection of topics and speakers that will make us all work smarter in response to an awareness of what is happening on the leading edge of forensic science.

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#### **BioMedical Engineer, Drug Intelligence, Aquatic Death/Abuse Investigations**



### W20 Genetic Genealogy: Science, Law, and Ethics

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**Learning Overview:** After attending this workshop, attendees will have a better understanding of how genetic genealogy works, how it is used by law enforcement, its admissibility in court, and the ethics of using personal DNA data of relatives to solve crimes.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by providing a clear understanding of the use of genetic genealogy to solve crimes and to better equip forensic scientists to appreciate the legal and ethical issues that it presents.

Since the identification and arrest of the suspect in the “Golden State Killer” investigation in early 2018, using novel DNA and genealogy tools, dozens of law enforcement investigators in North America have effectively used such methods in high-profile cold cases. A combination of advanced DNA methods, using thousands of Single Nucleotide Polymorphisms (SNPs) along with searching of ancestry databases has allowed genealogists to assist investigators in identifying possible persons of interest as suspects in unsolved crimes. These tools have significantly changed the face of genealogical searching and those changes now offer new investigative methods of crime solving.

Several DNA-based public genealogy databases can be searched for even distant relatives of those who leave crime scene DNA evidence, regardless of whether their profile is in a law enforcement database such as the Combined DNA Index System (CODIS). Investigators can submit the raw data files from the crime scene DNA profile to one of these new genealogy databases to help identify individuals who could be biological relatives of the person who left the evidence. While the suspect may not be in CODIS and have never submitted their DNA to any ancestry company, the DNA of relatives who submitted their own data for genealogy search purposes can lead police to a range of possible persons of interest. Confirmatory Short Tandem Repeat (STR) testing is then needed before any arrest is made.

This workshop includes a description of the DNA technology used, the database search process itself, and the methods of genealogy searching from expert leaders in those fields. The results of DNA-based genealogy searching still require careful data analysis and follow-up investigation by police to further narrow the range of possible persons of interest based on age, locale, and other factors associated with the crime. Law enforcement officials will describe how police investigators follow up on the possible suspect(s) generated by the genealogical research to obtain a current DNA specimen upon which to seek a warrant. The entire process may raise potential issues under the 4<sup>th</sup> Amendment, particularly considering the recent *Carpenter* United States Supreme Court opinions, as well as online privacy laws. These admissibility issues will be presented from both the prosecution and defense perspectives. There are also significant ethical questions presented by this process and those will be discussed by biomedical experts, particularly considering recent actions seeking to expand the “informed consent” for DNA contributors or even to legislatively prohibit the use of genetic genealogy to solve crimes.

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### Genetic Genealogy, DNA Genealogy, Genealogy Crime Solving



### W21 A Decade of Designer Drugs: Lessons Learned and Future Directions

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**Learning Overview:** After attending this workshop, attendees will be able to: (1) discuss current and past trends in Novel Psychoactive Substances (NPS); (2) describe and explain the means by which new substances are assessed for their potency and toxicity; (3) identify resources and strategies for the investigation of NPS toxicity outbreaks and impacts on user populations; (4) assess the available analytical techniques for resolving the identity of positional isomers of new drugs; and (5) apply best practices in the use of standard reference materials and proficiency testing for forensic NPS casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a ten-year perspective on how forensic and research sciences responded to the phenomenon of designer drugs, later NPS, the tools and workflows that have been developed, current approaches to evaluating drug toxicity, the impact of NPS on drug user behavior, and best practices for the future identification of structural identity and detail of newly emergent substances.

The emergence of novel stimulants mephedrone and methylene in 2007, followed by the first wave of synthetic cannabinoids in 2008, marked the outbreak of the “designer drug” phenomenon. Due to the number of terms used to refer to the different substances, including “legal highs”, K2, Spice, “bath salts,” and others, the scientific community agreed to refer to emergent substances as Novel Psychoactive Substances or NPS.

Attempting to agree on a universal term for emergent substances was the first of many challenges associated with NPS, and the field still struggles with consistent naming. The large number of substances are diverse in terms of their effects and chemistry, and this has created significant analytical challenges resulting from unavailability of standard reference materials and availability of the necessary technology. There have also been significant impacts on drug user practices and behaviors as a result of the changing market.

This workshop will begin with an overview of the challenges, successes, opportunities, and future threats posed by NPS and will introduce the tools and workflows that have developed to more quickly identify new substances and their potential harms, including *in vitro* and *in vivo* studies that help categorize new substances and their potency and toxicity, that are essential to decisions about scheduling these substances. Later presentations will cover examples of how investigators and laboratories have mobilized to respond to the investigation of NPS toxicity outbreaks and how the analytical data are used to support public health measures. Other speakers will provide perspective on how the lack of consistency in the drug supply have led users to change their behavior and take precautionary measures in their drug procurement and how successful or otherwise these strategies have been. While analytical methods are now becoming well established for many types of NPS detection, identifying the absolute configuration of the drug analogs is now receiving more attention, and the latter part of the workshop will assess emerging analytical techniques for resolving the identity of positional isomers of new drugs. The last part of the program will look at how the forensic community and their partners have addressed issues of rapid access to standard reference materials and expanding proficiency testing programs, including NPS detection.

#### Novel Psychoactive Substances, Designer Drugs, Emergent



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### W22 “All Rise”: Successfully Navigating the Judicial System as an Expert Witness

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**Learning Overview:** After attending this workshop, attendees will be well-versed in the decorum, procedures, expectations, and environment of a modern United States courtroom through active participation in the process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering real-world experience in expert testimony in an environment conducive to productive learning without the stress of an actual trial.

Forensic scientists are trained in the techniques, theory, history, and standards associated with their particular discipline. Often lacking, however, is training in the application of those skills to the courtroom setting and, in particular, the presentation of expert testimony. A forensic expert’s ability to effectively communicate in a courtroom setting is vital if that expert is going to make meaningful contributions to the justice system.

This workshop seeks to improve courtroom communication skills by providing a combination of lectures and hands-on learning that will introduce the basic foundations of the United States court system, the roles of the participants, the types of pre-trial and trial processes that occur, and the opportunity for attendees to practice testifying in a true-to-life courtroom environment with case-based scenarios.

Discussion will include the structure of the judicial system and the expert’s role in it. This brief introduction will be followed by a number of different legal proceedings that may be encountered by an expert witness. Pretrial hearings (such as *Daubert* hearings) and trial testimony are among the topics that will be explored. This workshop will be set up as a traditional courtroom with a judge, jury, court reporter, clerk of the court, and bailiff. Two experienced attorneys will perform direct and cross-examination derived from real-life scenarios. The attorneys will demonstrate common techniques used in court, such as focused direct examination, impeachment based on prior statements, personal interests or biases, and detailed cross-examination about the limitations of the discipline or analysis. Time permitting, the judge and jury will be allowed to ask questions as well (as occurs in many jurisdictions). The cases will all be criminal cases in order to provide the most commonly encountered scenarios in forensic science practice.

The goal of this workshop is to provide as many attendees as possible with a real-life courtroom experience. To that end, registrants will be selected to participate in the “trials,” “hearings,” and other legal proceedings. The jury will be peopled with registrants as well in order to provide real-time feedback, critique, and suggestions. All attendees will be actively engaged in the process in some way, with the end result that all will feel more comfortable and familiar with the courtroom. This workshop is geared toward scientists who have not yet testified, those that have limited experience, and those that have a lot of time at the Bar (the Bar, not the bar ...). This mix of experience will benefit all attendees and provide active, engaging interactions.

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#### Expert Witness, Judicial System, Testimony



### W23 Dispelling the Myths About the Forensic Examination of Handprinting

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**Learning Overview:** After attending this workshop, attendees will be informed about the development of manuscript handprinting, motor-control theories regarding how handprinting is learned, examination techniques with handprinting, and will conduct hand-on examinations of genuine and simulated handprinting.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by informing attendees on the history and development of manuscript handprinting and will enhance their abilities to conduct handprinting examinations. Attendees will also be prepared to defend the reliability of handprinting examinations in court.

Handprinting examinations have been conducted successfully for many years by Forensic Document Examiners (FDEs). However, there have been some recent court decisions in which handprinting evidence was deemed to be inadmissible due to a perceived lack of testing of the reliability of FDEs in handprinting examinations. This workshop will seek to dispel the myths created by these court decisions and statements of some critics of handprinting examination.

This workshop, which has a significant hands-on component, was designed to address the history and development of manuscript handprinting, the various styles of manuscript handprinting, and its fine and subtle elements. Research in handprinting together with casework examples will be presented. Motor-control theories of the development of handprinting will be presented and discussed. A review of empirical research conducted into the reliability of handprinting examinations will also be conducted.

Are there different examination techniques and methodologies applied to manuscript handprinting than applied to cursive writing? The transition from manuscript handprinting to cursive writing will be covered as will the similarities and dissimilarities between the different styles of handwriting.

Critics of the forensic identification of handwriting have now attempted to create an illusion that manuscript “handprinting” is not identifiable. As one critic stated recently, “Handwriting is different from handprinting as typewriting is different from either.” Unfortunately, some in the legal profession have accepted this absurd illusion, and the identification of manuscript “handprinting” is now separately under attack. One judge stated, “Typical handwriting analysis involves cursive writing, and the record is devoid of evidence that there is even a recognized field of expertise in the identification of handprinting.”<sup>1</sup> Another court opined, “Ultimately, the limited testing that exists is inconclusive as to the reliability of handprinting analysis.”<sup>2</sup> Recent court decisions and statements by critics of handprinting examination will be discussed and critiqued.

The issue of the techniques used to simulate manuscript handprinting and the difficulty factors in simulating cursive handwriting versus manuscript handprinting will be the main focus of this workshop, which will also include the study and examination of practical problems by the attendees.

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#### Document Examination, Handprinting, Reliability



### W24 Forensic Postmortem Radiology: Crossing the Border Between Radiology and Pathology

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**Learning Overview:** After attending this workshop, attendees will: (1) learn the basics in how to get started in the field of forensic radiology, (2) understand the current state of the art and levels of evidence in forensic radiology, (3) learn about applications to assist forensic pathologists and practitioners in medical death investigations, and (4) finally, will be made aware of potential resources available to interested practitioners.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with expertise from around the world on the use of best practices and the evidence base of forensic postmortem radiology.

It has been nearly ten years since the publication of the National Academy of Sciences Report calling for the strengthening of the forensic sciences. In their report, the National Academy of Sciences stated the critical role that imaging plays in the documentation of findings sufficient for courts, for providing the opportunity for review by outside experts, as well as for allowing for reevaluation of evidence as medical knowledge advances. Since then, forensic radiology and imaging has grown significantly as a field around the world.

The “virtual autopsy” or “virtopsy” utilizes Multislice Computed Tomography (MSCT) or Postmortem CT (PMCT) and Magnetic Resonance Imaging (MRI) combined with 3D imaging technology to create vivid images of the interior of the human body. CT scanning and MRI have been shown to present better visual pictures of some injuries, reduce the number of autopsies conducted to rule out occult injury, and document the extent of injury in accidents in greater detail. The advantages of the virtopsy are that it is not invasive or destructive to tissues and can provide clear pictures of skeletal and soft tissue injury. It also provides some information when there is a religious objection to autopsy. Additionally, virtopsy has the potential to detect internal bleeding, bullet paths, bone and bullet fragmentation, fracture patterns, brain contusion, and gas embolism, as well as occult fractures that are technically difficult to demonstrate during the traditional autopsy.

In the United States, only a few Medical Examiner’s (ME’s)/coroner’s offices (approximately 13) have access to this advanced medical imaging at this time and very few have the budget to purchase the expensive equipment or to build a suitable facility with staff to maintain it. The current public health crisis of opioid deaths is stretching an already strained ME’s system with regard to autopsy caps set by the National Association of Medical Examiners (NAME) standards. Postmortem medical imaging can play a critical role in easing these demands for full autopsy on offices through triage screening from PMCT.

Within the past year, the National Institute of Justice (NIJ) has fully supported forensic imaging as a supplement to the traditional autopsy and as a triage and long-term evidence documentation tool. NIJ efforts have attempted to bring field standards from around the world and establish major research areas of need in order to help facilitate the field’s growth in the United States since it lags behind other countries in the field. Additionally, the post-AAFS 2018 attendee survey indicated in several places the desire of members for more information on assisting forensic pathology workloads with the use of CT scans/documentation of trauma via imaging, even going as far as suggesting modifying of NAME guidelines to accomplish this. With the field well established in practice outside the United States, lessons learned from experts will help those interested in incorporating these technologies into their facility’s everyday practice. Exposure to emerging technologies in the field will also help practitioners be exposed to potential applications that will alleviate their workflow in an already overtaxed death investigation system.

This workshop will show how to start a program, give an overview of the current state of forensic imaging in the world, and demonstrate how future technologies may impact the field of forensics.

### Forensic Pathology, Forensic Radiology, Postmortem Computed Tomography



### W25 Working Toward a Wellness Mindset for Forensic and Investigative Personnel: Addressing Stress and Trauma in the Workforce and Taking Steps to Change Agency and Professional Culture

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**Learning Overview:** After attending this workshop, attendees will understand and recognize how stress and trauma are tied to the forensic professions, including in the form of vicarious trauma, and how exposure to trauma and stress impacts an employee's personal and professional performance.<sup>1-7</sup> Attendees will also learn evidence-based strategies to mitigate and reduce the impacts of stress and trauma, as well as learn from the experience of the anthropology program at the University of South Florida and the Maryland State Police's Crime Laboratory efforts to address stress and trauma. Attendees will be more aware of the various types of trauma that investigators and investigative personnel routinely encounter and the potential impacts this may have, not only on the individual, but on the investigation and/or service provision. This workshop builds upon and expands the content of the half-day workshop presented at the American Academy of Forensic Sciences Annual Scientific Meeting in 2019.<sup>8,9</sup> Paths forward and the implementation and incorporation of Evidence-Based Policy (EBP) will also be addressed so attendees can examine what policies and assistance are available in their own agencies or assist in policy development.<sup>10-18</sup> This workshop will address concerns related to both line-level and supervisory personnel.<sup>19</sup>

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by providing attendees with the understanding that the human experience is variable, and trauma exposure and symptoms even more so, leading to behavior patterns that can be complex and abstract and by recognizing the manifestations of a variety of psychological—both behavioral and cognitive—impacts as a reaction to trauma and stress exposure on the job. This workshop will also address the concept of trauma-informed agencies, ensuring that appropriate resources are available to employees, focusing on evidence-based interventions. By attending this workshop, attendees will be better equipped to understand the potentially short- and long-term impacts of trauma, as well as how to adapt investigative approaches and provide resources that will lead to increased chances of not only a positive work environment, but likely a more efficient and cost-effective solution to maintaining employee health and well-being to avoid burn-out and decreased cognitive abilities that may impact service provision and job performance.

In seeking to serve communities as forensic professionals, individuals are exposed to things the average citizen cannot understand; sometimes these experiences stay with the individual. Recognizing that trends (e.g., the opioid crisis) can exacerbate levels of stress and trauma faced by personnel, best practices suggest that a trauma-informed agency has made preparations for employees and will have better success in terms of employee resilience and productivity. This discussion is still in its infancy, due largely to issues of mental health stigma and the lack of research exploring how forensic personnel experience trauma.

This workshop aims to broaden and continue discussions on a topic often considered taboo and highlights the variety of traumatic experiences faced by personnel. Similar conversations have begun in different arenas associated with forensic sciences, evidenced by the creation of the American Society of Crime Laboratory Directors (ASCLD) Stress and Trauma Task Force, the development of the Forensic Technology Center of Excellence (FTCoE) webinar series, attention of the Organization of Scientific Area Committees (OSAC) Human Factors Committee, and mention in the forthcoming Department Of Justice (DOJ) Needs Assessment of Forensic Laboratories (NAFL) report.<sup>20-23</sup>

Cognitive implications subsequent to traumatic exposure are varied in presentation and require management, but may be difficult for forensic professionals to detect. Amnesic complications of dissociation, as well as shifts in cognitive schemas following trauma have implications on both cognitive functioning and possible treatment.<sup>24,25</sup> The cognitive impact of trauma can be seen in both primary and vicarious/secondary trauma exposure (e.g., peers, treating clinicians).<sup>26</sup> Some research has suggested a negative impact on executive functioning and memory, which may be compounded by repeated exposure to trauma.<sup>27-30</sup> These cognitive skills (necessary for effective forensic job performance) are being shown in the literature to be impacted by trauma exposure.<sup>31,32</sup>

Employee burnout can represent the loss of an employee, but also an agency's financial investment in individuals (e.g., training, development). This highlights the importance of striving for well-developed, empirically supported policies protecting the financial interests of the agency and jurisdiction, in addition to the well-being of personnel. Even if the employee impacted by stress/trauma does not leave the agency, they may represent a financial loss in terms of decreased productivity, workers' compensation, litigation, absenteeism, and other similar costs.<sup>33-35</sup> Professionals may not recognize the psychological impacts of trauma exposure or cumulative stress, and the easiest response may be identifying these impacts as an individual problem.<sup>36,37</sup> These effects may also relate to the human factors discussion taking place in the Academy.<sup>38-49</sup> Research suggests cost-effective approaches, and EBP should involve effective training, preparation, and swift intervention to avoid personnel developing longitudinal cognitive and/or psychological deficits following trauma exposures. Utilizing Seamone's argument to apply intervention and prevention programs, this workshop will discuss multiple EBP options, as well how appropriate training can have preventative effects.<sup>50-67</sup>

This workshop will help attendees identify factors associated with these aforementioned needs, in addition to implementing empirically supported strategies for proper management of cognitive/psychological concerns. This will impact the forensics community by elucidating that the human experience is variable (as are symptoms of trauma exposure), leading to complex and abstract behavior patterns. This workshop addresses the concept of trauma-informed agencies and ensuring that appropriate, evidence-based resources are available to employees. Attendees will be better equipped to



understand potential impacts of trauma, leading to increased chances of a positive work environment, increased efficiency, and maintaining employee well-being cost-effectively.

This workshop will use many cases, videos, and discussion points to illustrate the conceptual and applied understanding of how trauma and its impacts manifest. Given the nature of the material, this workshop is not recommended for those persons who are sensitive and/or in some form of crisis.

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### Stress/Trauma, Wellness, Resilience



### W26 Overcoming Analytical Challenges Inherent in New Psychoactive Substances With Gas Chromatography Coupled With Vapor Phase Infrared Detection (GC-IRD)

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**Learning Overview:** The goals of this presentation are to obtain: (1) a more comprehensive awareness and understanding of the analytical challenges inherent in the analysis of novel and emerging drugs; (2) an overview and better understanding of the role of GC-IRD applications in the analysis of seized drugs; and (3) knowledge and tools for the optimization of GC-IRD methodology for the analysis of opioids, synthetic cathinones, and synthetic cannabinoids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that GC-IRD is a useful alternative for the rapid detection and identification of novel substances when routine analytical techniques yield limited information.

After attending this session, participants will gain comprehensive knowledge and understanding on the unprecedented analytical challenges associated with the influx of novel psychoactive substances in the illicit market during the past several years. Synthetic substances, such as synthetic cannabinoids, opioids, and synthetic cathinones, continue to be illicitly manipulated to create more and new substances of equal or greater toxicity and potency.

With the emergence of these novel compounds, forensic crime laboratories are facing analytical challenges for the reliable detection and identification of these substances. Due to their structural similarities, routine analytical methodology (e.g., gas chromatography/mass spectrometry) is often not sufficient. To increase the complexity of the problem, a large number of seized drug evidence is now poly-drug, creating another challenge in the separation of these substances. Laboratories have had to explore other options to increase their scientific capabilities.

Forensic scientists are frequently finding themselves turning to GC-IRD as a suitable alternative to routine instrumental techniques. Understanding the theory and application of GC-IRD methods for the analysis of seized drugs will assist forensic scientists in better addressing the analytical challenges that are inherent with today's emerging psychoactive substances. Many of these substances generate the same or significantly similar mass spectra, present co-elution problems, or may have associated thermally degradable concerns.

While the technology of GC-IRD is not new, its application to the forensic analysis of seized drugs continues to increase. Attendees will be introduced to the most current analytical challenges associated with seized drugs analysis and will be introduced to GC-IRD background and theory. Attendees will learn how to optimize GC-IRD methods and apply them to overcome existing challenges associated with synthetic cannabinoids, opioids (specifically, fentanyl-related substances), and synthetic cathinones. Attendees will also learn how modern technological advances can be utilized for even complex mixtures, such as the volume programmable temperature vaporization inlet and light pipe temperature options.

An overview of the role of GC-IRD in forensic laboratories will be provided, and how its applications are increasing their capabilities and making them better equipped to address our nation's opioid crisis and influx of novel and unknown substances.

#### GC-IRD, Psychoactive Substances, Opioids



## A1 Collaborative Approaches in the Identification of Transgender and Gender Variant Decedents

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**Learning Overview:** After attending this presentation, attendees will have learned about a non-traditional, collaborative approach to aid positive identifications in unsolved cases involving transgender and gender variant decedents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) opening discussion about obstacles to positive identification of transgender victims; and (2) presenting a collaborative forensic anthropology/genealogy approach to assist in recovering and honoring the identities of these victims.

In cases involving skeletal remains, positive identification requires both osteological expertise and the ability to translate biological and contextual information into a culturally appropriate representation of gender identity expressed during life. Anthropologists are uniquely suited to address the issue of identifying transgender and gender variant individuals in forensic cases.

As osteological specialists, forensic anthropologists routinely estimate sex as part of the biological profile, often the first step toward identification. However, an increasing percentage of the United States population (up to 3.0% or 9.8 million people) identifies as transgender or gender variant, thus complicating the estimation of sex as a factor in positive identification.<sup>1</sup> Traditional sex estimation methods, while largely successful for cisgender individuals, may be insufficient for cases involving transgender individuals.<sup>2,3</sup>

A recent report demonstrates that as a result of systemic discrimination, transgender individuals experience disproportionately high rates of fatal violence and suicide.<sup>4</sup> This same discrimination also creates barriers to identification in death. Missing persons may be unreported or incorrectly reported because authorities or families refuse to acknowledge their gender identities.<sup>4</sup> Furthermore, transgender people may be mis-gendered on medical or legal documents on file; 68% do not possess identification that accurately reflects both their name and gender.<sup>4</sup> As a result, transgender Does may be described as either Jane or John, which may not match their lived gender or descriptions provided for them.

The Anthropology section of the American Academy of Forensic Sciences (AAFS) has actively worked toward increasing diversity and inclusion in the section and in anthropological research in recent years. However, the problem of identifying transgender and gender variant decedents remains underexamined in our field. This presentation focuses on one potential avenue toward identification resolution: collaboration with the Trans Doe Task Force (TDTF), an organization created to find and research cases involving transgender decedents to assist the non-profit forensic genealogy-focused DNA Doe Project. Both organizations are focused on generating positive identifications for John and Jane Does, though the TDTF focuses on researching and identifying Doe cases who were likely transgender or gender variant during life. Forensic genealogy is, in part, the application of genealogical research to the positive identification process. To date, AAFS does not recognize forensic genealogy as a forensic discipline due to an insufficient number of practitioners in its membership. This study posits that the TDTF is an important component of the growing conversation around challenges in identifying transgender victims in the field of forensic anthropology.

To illustrate the potentially productive collaboration between forensic genealogists and anthropologists, this presentation will present a case study in which a creative and collective approach to identification was pursued. The Doe involved was likely mis-gendered via forensic art and the original police investigation in the 1980s. The TDTF has identified this particular case as one that reflects the challenges surrounding cold cases involving gender variant decedents. The necessary steps in case identification are presented to illustrate a successful research process and submission to the DNA Doe Project. Traditional (e.g., the National Missing and Unidentified Persons System (NamUs)) and non-traditional means (e.g., archived LGBTQ+ zines, Trans Day of Remembrance lists, Facebook® pages dedicated to missing and murdered transgender victims, etc.) are used during case research; these factors will be of interest to forensic anthropologists who suspect they may be working on cases involving transgender individuals. Additionally, the case study will demonstrate why and how sex estimation methods should be augmented in cases of transgender victims. Finally, a discussion of the possible privacy concerns and case access issues involved in a combined forensic genealogy/anthropology approach will be reviewed.

In some cases, the translation of forensic genealogical data, in association with thorough police investigation and forensic sex estimation, are all necessary to resolve identities of transgender decedents. A thoughtful, culturally informed approach anchored by an appreciation of gender diversity and identity in marginalized populations will better serve the victim by more quickly resolving identifications and honoring the lived identity of the decedent.

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### Transgender, Sex Estimation, Positive Identification



## A2 The Frontal Versus Basal Region of the Cranium: A Comparison of the Best Sex Prediction Parameters Using Discriminant Function Analysis

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**Learning Overview:** After attending this presentation, attendees will understand the usefulness and methodology of sex estimation, especially from various measurements of the cranium, which will help attendees choose the preferred parameters for different regions of the cranium and enable them to conduct further research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting standards for sex estimation from various measurements of the cranial regions when the cranium as a whole or its parts, especially the frontal or facial and basal regions, is brought for forensic examination.

One of the primary goals of any medicolegal investigation of human skeletal remains is to ascertain its identity. To achieve this goal, estimation of the biological profile is the first step, which helps to narrow the area of search and potential number of suspects and, thus, helps in the medicolegal investigation. A biological profile includes estimation of age, sex, stature, and ancestry. Sex determination needs to be performed ahead of age, stature, and ancestry estimation as these factors are sex dependent. The cranium is among the most dimorphic parts of the skeleton, followed by the pelvis, when it comes to sex determination from skeletal remains. However, implications of factors such as age, stature, and secular trends in craniofacial morphology are yet to be understood and controlled completely and thus this variability in skull is so important that it has a direct bearing on the accuracy and reliability of forensic anthropology tools. These variations are population-specific due to their genetic, environmental, and socio-economic dependence. In this context, the present study was conducted to make discriminant function equations for determination of sex from the frontal and basal region of the cranium in a central Indian population. The study is based upon a random sample of 187 adult crania (116 male, 71 female) aged from 18 to 50 years. A total of 33 cranial measurements have been taken on each sample (24 on the frontal region; 9 on the basal region) according to the standard procedures. The data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) computer software. Sex differences in frontal and basal measurements were analyzed using Discriminant Function Analysis (DFA) and discriminant equations were calculated. The best parameters for sex prediction were estimated from various measurements of the two regions of the cranium using linear and stepwise DFA.

Statistically significant differences ( $p < 0.001$ ) were observed between males and females for most of the measurements of the cranium in both regions. A DFA was run on the measurements taken from both regions of the cranium separately. Mastoid breadth followed by bi-mastoid breadth proved to be the best assessors of sex among nine parameters of basal region when subjected to direct (80.7%) as well as stepwise (79.1%) DFA. However, in the frontal region, basion-bregma height followed by nasal height gave the best sex prediction among 24 parameters, with an overall accuracy of 81.8% with linear analysis and 78.1% when subjected to stepwise DFA. Height measurements showed higher degree of sexual dimorphism in the frontal region. However, the cranium showed maximum univariate sexual dimorphism in breadth parameters such as mastoid breadth and foramen magnum breadth. The present study shows that both the regions can be helpful in sex determination with almost comparable accuracy, the only difference being the dominant parameter in the frontal region is height dependent while that in the basal region is breadth dependent. Thus, in cases, where cranial fragmentary remains are from the basal region, breadth measurements should be preferred over other measurements and when only frontal parts of cranium are encountered, height measurements should be preferred over width measurements.

### Forensic Anthropology, Sex Estimation, Cranial Measurements



## A3 Sex Determination Through the Evaluation of Foramen Magnum Measurements on an Italian Population

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**Learning Overview:** After attending this presentation, attendees will understand a new system for sex determination using head Computed Tomography (CT) scan measurements of the foramen magnum.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new metric sex determination for Caucasians from ages 18 to 90 years.

This method is applicable for complete remains (cases in which it is comparable to other parameters) and incomplete ones due to dismemberment, trauma (with no affection of the cranial base), and advanced states of decomposition.

Sex determination is an important initial step in forensic identification of unknown skeletal remains and it could be reached in different ways. One of these, less described, is the morphometric evaluation of the foramen magnum. A few studies have been published, both on dry skull and on CT scan evaluations. In the literature, CT scan measurements of the foramen magnum have been obtained on Turkish, Swiss, South Indian, and Iraqi populations, but no study has been conducted on an Italian population. Furthermore, measurements taken into consideration in the present study were used in only one study on a Saudi Arabian population.<sup>1</sup>

The CT images included in the present study were obtained from Caucasian patients undergoing head CT for medical or surgical reasons. The sample was composed of 50 adults, 25 males and 25 females. Subjects with congenital or acquired cranial deformities were excluded. Measurements were made using Horos™ Software Version 3.0. The following measurements of the foramen magnum were taken: Length of the Right Occipital Condyle (LROC), Length of the Left Occipital Condyle (LLOC), Width of the Left Occipital Condyle (WLOC), Width of the Right Occipital Condyle (WROC), Maximum Bicondylar Distance (MBD), Length of the Foramen Magnum (LFM), Width of the Foramen Magnum (WFM), Area of the Foramen Magnum (AFM), and Minimum Bicondylar Distance (MiBD).

The parameters 1 to 8 were evaluated from the Saudi Arabian study; the MiBD was a new parameter chosen for a more complete evaluation of bicondylar distances.<sup>1</sup>

The primary purpose of this study was to validate the use of Saudi Arabian measurements even on an Italian Caucasian sample. Another goal was determining whether the new measurement (MiBD) could also be used in sex determination. In the Saudi Arabian study, concordance was assessed for sex with LROC, LLOC, LFM, WFM, and AFM. In this study, results from preliminary statistical analysis suggest a statistical association of sex with LROC, LLOC, LFM, WFM, and AFM. Furthermore, no statistically significant concordance was found between sex and this new parameter (MiBD).

In conclusion, results were encouraging; therefore this study's goal is to broaden the sample in order to obtain more statistically significant results. Furthermore, this method could be reliable and repeatable and may provide forensic anthropologists with an additional tool for sex determination, especially in situations where remains do not include other bones, especially the long ones.

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### Anthropology, Sex Determination, Foramen Magnum



## A4 Assessing the Utility of Vertebral Body Heights as a Sex Indicator in United States Whites and Koreans

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**Learning Overview:** After attending this presentation, attendees will understand that the vertebrae have a potential to be used for sex estimation in United States Whites and Koreans. However, their utility differs between the two populations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by testing for the utility of United States White and Korean vertebral body heights as a sex indicator, which has not been studied previously. Sex estimation using the vertebrae will enhance the likelihood of identification of unknown skeletal remains, particularly when other sex indicators are missing or damaged. Additionally, this study emphasizes that the vertebrae-based sex estimation methods need to be population-specific.

Sex estimation is a crucial part of a biological profile in forensic anthropology. The cranium and pelvic bones are regarded as the most useful sex indicators. Other parts of a skeleton are also used for sex estimation, mostly due to size differences between sexes. The vertebrae have been studied for this purpose, but it has been reported that the accuracy of sex estimation using the vertebrae may vary in different populations.<sup>1</sup> This study explores how significant the contributions the vertebrae can make to sex estimation are in United States Whites and Koreans.

Vertebral body heights from C2 to L5 were measured from 140 Koreans (75 males and 65 females housed at eight institutions in South Korea) and 102 United States Whites (51 males and 51 females at the Bass Donated Skeletal Collection of the University of Tennessee) following Raxter et al.<sup>2</sup> Then, the discriminant function analyses were conducted on the individual vertebrae, followed by the stepwise analyses to find a set of the most influential vertebrae.

Discriminant equations using the Korean samples yielded decent-to-good Correct Classification Ratios (CCRs) ranging from 65% (L2)–92.1% (stepwise analysis). The CCRs of 75% or greater were obtained from 19 out of 24 equations (i.e., equations with the C2–T11, L1, and stepwise analysis). On the other hand, the performance of the equations using the United States White samples was poorer, with the CCRs ranging from 48% (L2)–81.4% (stepwise analysis). Only four equations (i.e., equations with the C4, C6, T2, and stepwise analysis) yielded the CCRs of 75% or greater. For both the United States Whites and Koreans, the lumbar-involved equations tend to yield lower CCRs, except for the Korean equation with the L1.

In this study, the United States males (176.2cm) and Korean males (162.2cm) are taller than the United States females (163.3cm) and Korean females (148.4cm) by 12.9cm and 13.8cm, respectively. The summed vertebral body heights correspond to approximately 30% of the total stature in both populations. In Koreans, the difference in the summed vertebral body heights between sexes (4.2cm) explains 30.3% of their stature difference; however, in the United States Whites, only 20.2% of the stature difference can be explained by the difference in the vertebrae (2.6cm). These findings indicate that the vertebral column of the United States Whites does not contribute to the stature difference as much as that of the Koreans. Poorer performance of the United States discriminant equations may also be explained by the smaller differences in the vertebral body heights between the United States males and females. Vertebral body heights have potential as a sex indicator. However, their utility appears to depend on the biological characteristics of the target population. Therefore, if the vertebrae are used for sex estimation, population-specific methods need to be generated and validated before use.

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### Sex Estimation, Vertebrae, Population Specific



## A5 Estimation of Sex Based on Postcranial Elements in North American and Latin American Populations

Sara C. Zapico, PhD\*, Florida International University, International Forensic Research Institute, Miami, FL 33199; Joe Adserias-Garriga, DDS, PhD\*, Mercyhurst University, Erie, PA 16546

**Learning Overview:** After attending this presentation, attendees will have gained a better understanding of the potential use of postcranial elements for sex determination in North American and Latin American populations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the usefulness of the ulna and humerus for sex estimation with high accuracies.

Sex estimation is one of the first components to be assessed toward the determination of the biological profile in skeletal remains. Classically, the visual analysis of the pelvis is the preferred indicator of sex with a high degree of reliability. However, different scenarios render incomplete skeletons, leading to the examination of various parts of the body and the application of different techniques for the correct assessment of this parameter.

Although different studies pointed out that the skull is the second best estimator of sex through visual assessment, when applied to statistical models, it was not possible to reach the same accuracies as the pelvis. In fact, some studies indicated that postcranial sex estimates are generally superior to skull estimates. Consequently, several studies have been focused on the analysis and measurement of long bones toward this purpose, including, in some of them, the clavicle and scapula, and on increasing the accuracies of sex estimation. Although these studies indicated an improvement in sex estimation when using postcranial elements, an issue is the scarce work on Latin American populations, since the majority of these studies were developed on African- and European-Americans.

Previously described metric traits (maximum length of clavicle; cranial-caudal diameter of clavicle; diameter of humerus head; humerus condylar breadth; scapular height; scapula breadth; femur epicondylar breadth; and ulna minimum breadth of the olecranon) and a new one (olecranon-coronoid distance from ulna) were measured from 72 North American individuals (41 males and 31 females) and 59 Latin American individuals (32 males and 27 females) from the American Museum of Natural History Collections in New York City and the Texas State University Collections in San Marcos, TX. Statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 15. Comparison between groups was conducted using parametric or non-parametric tests. Discriminant function analysis models were performed to develop specific formulas for sex estimation.

Three independent measurements in three independent days were taken. After statistically corroborating that there was not intra-observer error, the average of these three independent measurements was calculated and used to analyze their significance between males and females. In both populations, all parameters showed significant differences between these two groups. However, when these measurements were introduced into discriminant function analysis in the North American population, only the minimum breadth of the olecranon correctly classified 91.3% of individuals and through cross-validation 89.9% of individuals. When this parameter was combined with the diameter of the humeral head, 98% of individuals were correctly classified and 98% through cross-validation.

In the Latin American population, the introduction of all parameters into discriminant function analysis retrieved the same results, although with lower classification accuracies. Again, the minimum breadth of the olecranon provided the highest classification accuracies (82.4% of individuals) and through cross-validation (80.4%). In contrast, in this case the diameter of the humeral head correctly classified 79.4% of individuals, with the same through cross-validation. This discrepancy with respect to the North American population could be due to the reduced number of analyzed individuals (71 vs. 59).

These results are in agreement with previous studies, which pointed out the humerus as one of the potential bones to take into consideration for sex estimation based on its accuracy. In addition, the minimum breadth of the olecranon in the ulna showed a high accuracy toward this purpose, rising in combination with the diameter of humeral head, at least in the North American populations. Thus, these two anatomical regions could be used alone or in combination with other methodologies for sex estimation, which is particularly important in situations of fragmentary remains. Future research may be able to expand on these results, increasing the number of individuals to improve the estimation accuracies.

### Postcranial Elements, Sex Estimation, Ulna





## A6 Evaluating Sexual Dimorphism Among South African Groups Using Dentition

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**Learning Overview:** After attending this presentations, attendees will have gained knowledge of sexual dimorphism in dentition among South African groups and the use of dentition, particularly canines, for sex estimation. Sex estimation is an important component of the biological profile as other parameters, such as age-at-death, ancestry, and stature, are dependent on it. This presentation illustrates how teeth are a good source of information on sex for extremely fragmented remains, owing to their postmortem longevity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the application of knowledge of sexual dimorphism among three major South African groups. A more in-depth knowledge of sexual dimorphism within and between South African populations can assist in the creation of biological profiles for unidentified persons with the use of large dental databases and statistical software, as well as for improving dental implants and orthodontic treatment.

Human variation is a core component of an accurate biological profile from a heterogeneous population. South Africa is a poly-linguistic society with more than 49 million people and is ideal for evaluating human variation, particularly sexual dimorphism, among diverse populations. Within the last 500 years, various groups, such as the Dutch, French, Malaysian, and Indian, have migrated to the country. Apartheid segregation laws affected gene flow among indigenous and migrated groups, which contributed to distinct social/political designations including Black, White, "Colored," and Indian South Africans. Today, approximately 80% of the population identify themselves as Black; 9% "Colored"; 8% White; and 3% Indian. "Colored" refers to a social group primarily from the Western Cape who are descendants of slaves from Indonesia, India, Malaysia, and Asia, and who were mixed with Europeans and indigenous Khoi and San. The purpose of this study was to examine sexual dimorphism among the dentition of modern South African populations and to assess whether observed dental variations were useful for developing population-specific sex estimation formulae for Black, "Colored," and White South Africans.

All available teeth (excluding third molars) were used from 906 adult crania with a known demographic profile (526 males, 380 females, 318 Black, 312 White, and 276 "Colored" South Africans). Data was obtained from three large modern 20<sup>th</sup>-century skeletal collections in South Africa (the Pretoria Bone Collection, the Raymond A. Dart Collection, and the Kirsten Skeletal Collection). Four permanent crown dimensions were taken: maximum mesiodistal, maximum buccolingual, and two molar diagonal diameters (mesiobuccal-distolingual and mesiolingual-distobuccal).<sup>1</sup> Technical Error of Measurement (TEM), Analysis of Variance (ANOVA), sympercents, and Linear Discriminant Analysis (LDA) were computed in R (R. Core Team, 2014) to describe the data and to generate population-specific sex estimation formulae.

All dental dimensions are highly repeatable with low inter- and intra-observer errors. All dimensions show statistically significant differences between sexes, with males possessing larger dimensions than females. All dimensions and all groups exhibited sexual dimorphism. Canines had the highest degree of sexual dimorphism, followed by molars, premolars, and incisors. Black and "Colored" South Africans presented with more sexual dimorphism than White South Africans for all variables. LDA provided cross-validated correct classification rates up to 87%. Mandibular models performed best for Black South Africans (79.45% to 87.50%) and White South Africans (60.71% to 85.71%), while models including upper and lower canines and premolars performed best for "Colored" South Africans (73.68% to 83.33%).

Despite much research into sex and ancestry estimation among South Africans, less attention has been paid to human variation in the dentition within and among these groups.<sup>2</sup> While all teeth were sexually dimorphic, the best multivariate combinations yielded accuracies similar to those of the cranium (<90%). The long bones, scapula, and pelvis outperform the skull and dentition.<sup>3</sup> In any forensic investigation, the pelvis and long bones, respectively, take preference over teeth. However, the dentition can be useful in circumstances of extreme fragmentation, burning, and/or commingling.

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### Linear Discriminant Analysis, Human Dentition, Biological Profile

## A7 The Relationship Between Soft Tissue Anatomy and Skeletal Sexual Dimorphism in the Cranium and Clavicle

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**Learning Overview:** After attending this presentation, attendees will understand the relationship between muscle, ligament, and skeletal enthesis size as it relates to human skeletal sexual dimorphism in both the cranium and clavicle.<sup>1-3</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying how soft tissue affects the morphology of the underlying skeleton and how this relates to sexual dimorphism at three skeletal landmarks: the nuchal crest and the mastoid processes of the cranium, and the rhomboid fossa of the clavicle. Additionally, the forensic science community will have the opportunity to discuss future directions for establishing a metric sex estimation method from the dissection phase of this study, which will be the focus of this presentation.

The overall goal of this research is to explore ways of establishing accurate metric sex estimation methods from the cranium and clavicle, through the dissection of donated human cadaveric material and analysis of skeletal and soft tissue sexual dimorphism. In forensic anthropology, an absent pelvis requires sex estimation from other areas of the skeleton, and many morphological and metric methods are available.<sup>1-4</sup> However, morphological sex assessment can sometimes lack reliability due to the subjectivity and experience required for scoring skeletal morphology.<sup>5-8</sup> It is important to have accurate sex estimation methods to help with identification in modern forensic cases and to understand populations in archaeological settings. Metric methods can provide not only accuracy, but also limit the subjectivity and expertise that is required for morphological methods.<sup>4</sup> This is a multidisciplinary research project, which approaches a forensic anthropology question from an anatomical perspective.

This presentation focuses on the dissection phase of this project and the analysis of soft tissue associated with sexually dimorphic skeletal landmarks. Relevant soft tissue attaching to the nuchal crest includes the upper trapezius (distal limit at the clavicular attachment), semispinalis capitis, and the nuchal ligament. Muscles of interest for the mastoid processes are the sternocleidomastoid, splenius capitis, and longissimus capitis. Soft tissue related to the rhomboid fossa includes the clavicular head of the pectoralis major, sternohyoid, subclavius, and the costoclavicular ligament.

A total of 22 bequeathed cadavers from modern European New Zealand and Thai populations were dissected using standard dissection equipment. Right and left sides of each element, where available, were dissected for a total of 406 elements: 352 muscles and 54 ligaments. Muscles were dissected by individual fascicles, which were measured for length and weighed for mass (converted to volume, per muscle density constant: mass/1.0576g/cm<sup>3</sup>). This was used to calculate the physiological cross-sectional area, or overall size, of the muscle (fascicle volume/length). Ligaments were measured for cross-sectional area (overall volume/length) and assessed separately from muscles.

Skin and fascia were removed to expose the relevant superficial muscles: the upper trapezius, sternocleidomastoid, and pectoralis major. Following their dissection, further muscles, such as the rhomboid major and minor, were removed to expose the deeper muscles: the splenius capitis, longissimus capitis, and semispinalis capitis. After the removal of each muscle, a grease pencil was used to outline all areas of attachment on the skeleton, providing the footprint of the entheses.

A 3D scan of the specimens was then taken, using a 3D scanning tablet attachment, to measure the total area of each enthesis as they related to the muscles and ligaments, as well as the skeletal landmarks with which they are associated. Following this, the sternum, clavicles, and partial first ribs were removed en bloc to access the sternohyoid, subclavius, and costoclavicular ligament for dissection. After all muscles and ligaments were dissected and recorded, the remaining elements were 3D scanned. Finally, the skull, vertebral column, clavicles, and sternum/manubrium were prepared for maceration for future analysis of the dry skeleton.

Preliminary results indicate that males show generally larger muscles than females, with some exceptions. Following standard methodology, the individuals are consistent with the male and female skeletal morphology.<sup>1-3</sup> Even muscularly robust females show standard female characteristics, skeletally. The implications for this muscular data can provide further insight into human sexual dimorphism in these well-understood skeletal landmarks, in addition to the lesser-understood sexual dimorphism of the neck muscles. Future development of a population-specific metric sex estimation method from these data will contribute to improving the accuracy of sex estimation from the cranium and clavicle.

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### Forensic Anthropology, Sexual Dimorphism, Muscle Size

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## A8 Sexual Dimorphism in the Shape of the Auricular Surface of the Ilium

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**Learning Overview:** The goals of this presentation are for attendees to: (1) develop a better understanding of the factors influencing the shape of the auricular surface; and (2) recognize the limitations of using the shape of the auricular surface to determine the sex of the decedent from skeletonized remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a statistically rigorous study of the shape of the auricular surface using a large recent skeletal sample, helping to resolve the contradictory findings of prior research.

The following null hypotheses are tested: the shape of the auricular surface does not covary with any independent (profile) variables, including: (1) sex, (2) age, (3) collection of origin, (4) body size, or (5) the interaction between sex and collection of origin.

The coxa is considered to be the most sexually dimorphic bone in the adult human body and one of the most useful for sex determination from the skeleton. The auricular surface of the ilium preserves well and is often present in archeological assemblages and forensic cases. While the shape of the auricular surface has been analyzed with both metric and non-metric techniques, these studies tend to simplify the feature's complex shape or else rely on the use of a number of arbitrarily defined landmarks, which can limit the researcher's ability to describe subtle shape differences or to elucidate the causal effects of independent variables.

In this current study, the auricular surfaces of 964 identified "White" individuals from the United States (Bass Collection and Hamann-Todd Collection) and Portugal (Coimbra Collection) were photographed, outlined, and subjected to elliptical Fourier analysis and principle component analysis. Analysis of Covariance (ANCOVA) was then applied to the resulting eigenvalues for each principle component to tease out the effects of each independent (profile) variable on auricular shape. Five effective principle components were distinguished. The first principal component (PC1) explains ~38% of the variation in the shape of the auricular surface and reflects the posterior extension and curvature of the posterior border; it is not influenced by any of the tested independent variables and may reflect difficulty in accurately locating the vaguely defined posterior edge of the surface. PC2 explains ~18% of the variation in the shape of the auricular surface and depicts what has traditionally been described as its "C-shaped" or "L-shaped" appearance. PC2 is significantly affected by sex, with male surfaces being straight and "L-shaped" and females being more curved and "C-shaped".

The stature of the decedent is also significant in females, where taller females tend to have more "feminine" auricular surfaces. PC3 explains ~14% of the variation in shape and describes the pinching of the caudal limb and whether the center line running through the apex is twisted, creating an asymmetrical appearance. Females have a more pinched caudal limb and the apex is oriented toward the cranial limb (asymmetrical), while males have a less pinched caudal limb and the apex is oriented directly above/in front of the posterior indentation (symmetrical). The collection of origin is a significant influence, with individuals from the Bass Collection having less pinched caudal limbs than individuals from the Coimbra or Hamann-Todd Collections. PC4 explains ~6% of the variation in shape and describes the thickness of the cranial limb relative to the caudal limb. Only the collection of origin was found to influence the shape variation described in PC4, where the bulbous cranial limbs in the Bass Collection differ from the thinner cranial limbs of the other groups. PC5 explains ~5% of the variation in shape and describes whether both limbs are pointed or bulbous and whether there are one or two indentations in the posterior border. Again, only the collection of origin was found to influence the shape, where the double indentation of the Bass Collection differs from the single indentation of the other groups.

Overall, the best predictor of the shape of the auricular surface is the sex of the individual, but this factor only accounts for ~0.6% of the total shape variation as described by the effective principal components. Therefore, while the traditional understanding of sexual dimorphism in the shape of the auricular surface is verified here, anthropologists should give priority to other pelvic features over the auricular surface when determining the sex of a decedent.

### Forensic Anthropology, Sexual Dimorphism, Auricular Surface



## A9 Application and Evaluation of Adult Morphological Sex Traits Using the Subadult Innominate

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**Learning Overview:** After attending this presentation, attendees will understand the age at which adult morphological sex traits of the innominate can be applied to subadults without compromising accuracy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that the innominate can be used to estimate the sex of individuals younger than previously thought possible.

Sex estimation is arguably the most important parameter of the biological profile, as accurate estimates of age, ancestry, and stature are often contingent on sex. Morphological evaluation of the ventral arc, subpubic contour, and medial aspect of the ischio-pubic ramus, as originally described by Phenice and later modified by Klales et al., has shown to consistently provide accurate estimates of sex when applied to adults.<sup>1,2</sup> However, Phenice has warned against applying these traits in individuals younger than 20 years of age.<sup>1</sup> Further, it is fairly ubiquitous among anthropologists that sex indicators are not valid and/or sex estimation is not applicable in individuals less than 18 years of age. Indeed, attempts to estimate sex in subadults have routinely yielded low classification accuracies and, therefore, it is advised against, especially in forensic contexts, for fear of misidentification. Unfortunately, this has led to a paucity of research on the subject and stagnation in contributions to this area of the field.

To evaluate the performance of these three traits when applied to subadults and to determine the age at which adult levels of accuracy are achieved, the Klales et al. method was applied to a sample of 301 individuals aged between 8.29 and 20.96 years using Multi-Slice Computed Tomography (MSCT) postmortem scans.<sup>2</sup> Age cohorts were created using two-year intervals for individuals 11 years and older, and a three-year age cohort was created for individuals aged 8.29 to 10.9 years to help increase the comparatively small sample sizes for younger individuals. Fisher's exact test with Monte Carlo simulation was used to test for sexual dimorphism in trait score frequencies between subadult males and subadult females for each trait in each age cohort. To determine at which age trait score frequencies are comparable within the sexes (e.g., subadult and adult females), subadult score frequencies for each cohort were compared to adult score frequencies previously reported in a comprehensive global study.<sup>3</sup> A Kruskal-Wallis test was applied and if rejected, a Dunn's test with Holm's stepwise adjustment was used to determine which age cohorts differed.

Significant differences in score frequencies between subadult males and females were observed for all traits and all age cohorts ( $p < 0.001$ ) with the exception of subpubic concavity for the 8 to 10.9 year cohort ( $p = 0.827$ ). Score frequencies between subadult and adult males differed in all cohorts, but only for the medial aspect of the ischio-pubic ramus ( $p \leq 0.018$ ). Differences in score frequencies between subadult and adult females were more common than in males and significant differences were observed in all traits in all cohorts ( $p \leq 0.01$ ), with some exceptions in older cohorts. Subadult accuracy rates for combined sexes showed an extreme bias toward male classification for the 8 to 10.9 year and 11 to 12.9 year cohorts, with males achieving perfect accuracies and females achieving 55.6% and 66.7% accuracies, respectively. A notable change was observed in the 13 to 14.9 year cohort, with males achieving an accuracy of 92.9% and females achieving an accuracy of 86.4%. By 15 years, both sexes in all remaining cohorts classified at rates at or above 90%, with sex biases switching to favor female classification. The accuracies achieved by 15 years are comparable to those obtained for adults, and sex biases in favor of female classification are consistent with results observed in adults.<sup>2,3</sup>

The results of this study suggest that adult morphological sex traits of the innominate can be used to estimate sex in individuals as young as 13 years, though comparable performance of the indicators are not observed until 15 years of age. The findings suggest sex can be estimated prior to individuals reaching skeletal maturity, which is minimally five years younger than previously advised. While these results are encouraging, it is recommended that more research be conducted to corroborate these findings before widespread application of the Phenice/Klales et al. methods to subadults in forensic casework.<sup>1,2</sup>

*This research was funded in part by a National Institute of Justice (NIJ) grant.*

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### Sex Estimation, Innominate, Subadults



## A10 Sex Estimation of the Subadult Pelvis Prior to Acetabular Fusion

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**Learning Overview:** After attending this presentation, attendees will understand the limitations in sexing the subadult pelvis prior to fusion of the acetabulum.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing sex estimation results and recommendations for ilium outlines and basic pelvic measurements on a large sample of modern United States subadults.

The Scientific Working Group for Forensic Anthropology (SWGANTH) suggests that sex estimation should not be performed on subadults less than 12 to 14 years of age. A review of the literature, however, reveals multiple studies on the subadult pelvis that report significant levels of sexual dimorphism and suggest a potential utility for subadult sex estimation, particularly using variables related to the ilium. The goal of this study was to test the ability of ilium outlines, greater sciatic notch measurements, and pelvic length indices to differentiate males from females in a large, modern sample of subadults.

Pelvic 3D surface models were extracted from postmortem Computed Tomography (CT) scans of 392 subadult individuals obtained from the University of New Mexico Health Sciences Center, Office of the Medical Investigator aged 0 to 14 years. Only individuals in which the tri-radiate cartilage of the acetabulum remained unfused were included in the study. Once orientation was standardized, 2D images of the internal/visceral surface of the ilia were extracted from the 3D models, and elliptical Fourier analyses were performed on the ilium outlines (contours), followed by a Principal Component (PC) analysis. Pubic length, ischial length, iliac height, maximum ilium breadth, and minimum ilium breadth were collected from the virtual specimens, as were five measurements from the greater sciatic notch. The PCs and various indices of the pelvic and greater sciatic notch measurements were subjected to Multivariate Analysis of Variance (MANCOVA) analyses to test for sex differences given age as a covariate. Discriminant Function Analyses (DFAs) were performed to evaluate correct sex classification rates. It was hypothesized that sexual dimorphism in the pelvis should increase with age; thus, analyses were rerun systematically eliminating the youngest one-year cohort one at a time in an attempt to determine a minimum age at which the subadult pelvis can be sexed.

MANCOVA results indicate significant sex differences in ilium outline shape and pelvic measurements ( $p < 0.05$ ), and Analysis of Covariance (ANCOVA) results revealed sexually dimorphic features related to greater sciatic notch shape and relative pubic length. Despite significant sex differences, less than 70% of the individuals could be sexed correctly with the DFAs, regardless of the variables included. Eliminating the youngest cohorts did not improve results. Scatter plots illustrate a large overlap between males and females in all variables. These results highlight the fact that statistically significant sex differences do not equate to forensically acceptable classification results. Previous studies may be confounding those results or their sample biases may be contributing to their positive results. When limiting analyses to only those individuals over the age of five years in the current study, sample sizes were greatly reduced; thus, additional samples and analyses should be performed to evaluate potential sex classification in subadults over the age of five years. Individuals aged five years or less, however, do not have high enough levels of sexual dimorphism in overall ilium shape, greater sciatic notch morphology, or pelvic length indices to have utility in forensic anthropological assessments. Age was a significant covariate for almost all variables, and morphological variation related to growth and development likely obscures any potential sex-discriminating variables at these young ages.

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### Sex Estimation, Juvenile, Os Coxae



### A11 3D Models of Paranasal Sinuses to Establish Age, Sex, and Ethnicity Across Three Modern Populations

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**Learning Overview:** The objective of this research is to assess if the shape or size of the paranasal sinuses provide patterns that distinguish between age, sex, and ethnicity on unknown remains that may assist with victim identification in forensic reconstructions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community as attendees will leave with an understanding of the production, accuracy, and utility of Three-Dimensional (3D) modeling as a primary visualization technique for 3D entities and the ability of using this method for human identification. Attendees will be introduced to the findings of preliminary research that highlights the potential of 3D modeling as a novel method for examining the paranasal sinuses to identify patterns that may assist with distinguishing between individuals and victim identification.

The identification of unknown human remains can be an essential element to a forensic investigation or crime reconstruction. Technological advances have furthered the development and understanding of trace evidence such that DNA and fingerprints have become the foundation of human identification. However, when a body undergoes extreme damage, such as in cases of arson, these methods of identification may not be possible. In conditions such as these, alternative methods of identification become critical.

Previous research has quantified the variability of the frontal sinus between individuals and has likened the distinctiveness of this structure to that of fingerprint comparison. As a result, the frontal sinus has been successfully used as a primary source for identification supported by expert testimony in the court of law. Therefore, examining the effectiveness of the paranasal sinuses in determining age, sex, and ethnicity is a valuable next step. However, the existing literature on the paranasal sinuses are targeted toward practicing physicians for medical care and not explicitly intended for human identification in a forensic context. For example, morphological patterns of the ethmoid sinus between populations has been documented solely as a precautionary guide to mitigate complications during endoscopic sinus surgery. Consequently, the ability of the paranasal sinuses to provide biological information to assist with forensic identification is under-researched. Therefore, there are no standard approaches for measuring or analyzing these structures.

This study addressed this gap by developing a new approach for human identification using 3D models of the paranasal sinuses. Models were produced from a database of modern Computed Tomography (CT) scans provided by University College London Hospital (UCLH). Elliptic Fourier and linear analysis of 30 3D models produced from the CT data demonstrated notable variations and patterns with regard to discriminating age, sex, and ethnicity across three distinct ethnic groups. The most promising classification rates ranged from 93% ( $p=.000$ ) to 70% ( $p=0.05$ ). This study also aimed to assess the correlation between these variables to determine if one particular sinus has a more successful prediction rate than others to assist with identification.

The findings from this study lay the foundations for developing the means for fast and reliable identifications to be made as a result of computer-assisted methods based from 3D reconstructions of the paranasal sinuses.

#### Identification, Three-Dimensional Reconstructions, Paranasal Sinuses

## A12 Assessing the Accuracy of Current Ancestry and Sex Estimation Methods on a Japanese Sample

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**Learning Overview:** After attending this presentation, attendees will have gained a better understanding of the accuracy of current ancestry and sex estimation methods on a Japanese skeletal sample.

**Impact on the Forensic Science Community:** This study will impact the forensic science community by investigating the suitability of currently available reference samples. As these reference samples serve as the basis to estimate ancestry and sex within a medicolegal context, it is critical to understand their accuracy in estimating these parameters on known skeletal samples. This work also highlights the diversity present in skeletal populations and the need for large diverse reference samples.

According to the Migration Policy Institute, from 1960 to 2014 there was a 2,597% increase in Asian migrants to the United States, thus representing 30% of the United States' foreign-born population.<sup>1</sup> Further, the 2010 Census reported that the Asian population is the fastest growing of any population in the United States. Specifically, the Japanese population experienced an increase of up to 59%.<sup>2</sup> However, most current biological profile estimation methods in the United States are based on individuals of European and African ancestry, which are then used to estimate the biological profile of unknown decedents in forensic cases. Despite studies stating the need for population-specific biological profile estimation methods, there is a scarcity of research that has focused on ancestry and sex estimation methods for Asian populations. As Asian migration into the United States continues to rise, so will the necessity for Asian-specific methods and reference data.

Heritable traits under investigation in biological anthropology include four major data types: craniometrics, cranial non-metrics and macromorphoscopies, odontometrics, and dental morphology. Each data type reflects different biological, environmental, and evolutionary influences. While similar research projects typically utilize only one or two data types, this study includes all four data types in a single project to present a holistic approach to analyzing ancestry and sex estimation methods on an Asian sample. The aims of this study are: (1) to test the accuracy of current ancestry and sex estimation methods on a Japanese sample, and (2) to explore the applicability of available reference populations when estimating the biological profile of unknown Asian decedents.

Data were collected from a modern Japanese sample curated at the Jikei University School of Medicine in Tokyo, Japan ( $n=33$ ). Data for each individual were input into various statistical estimates typically used by forensic anthropologists. Craniometric data were assessed using FORDISC® 3.1 to estimate sex and ancestry.<sup>3</sup> Cranial non-metric and macromorphoscopic data were analyzed using tetrachoric Mahalanobis distance and visualized using multidimensional scaling to assess population affinity.<sup>4</sup> Odontometric data were compared to data presented in Pilloud et al. using FORDISC® 3.1 to estimate sex and ancestry.<sup>5</sup> Dental morphological data were input into the statistical program rASUDAS to estimate ancestry.<sup>6</sup>

Preliminary results using the craniometric, odontometric, and dental morphology datasets produced correct classification rates from 21.10%–36.40% for ancestry, and the craniometric and odontometric datasets produced correct classification rates from 24.20%–36.40% for sex. The craniometric dataset produced the most accurate estimates of ancestry, while the odontometric dataset provided the most accurate sex estimates.

These varied results likely stem from inappropriate or insufficient reference samples, unexplored variation in the study sample, and/or the statistical treatments used in the current ancestry and sex estimation methods. Within forensic anthropology, there is a need to establish more robust reference samples that represent a broad range of global variation. It may also be necessary to explore additional statistical treatments and the incorporation of more than one type of data to accurately quantify population variation and sexual dimorphism.

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### Ancestry Estimation, Sex Estimation, Asian Population



## A13 Crossing the Borders of Linguistics, Ancestry, and Race in the Field of Forensic Anthropology

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**Learning Overview:** The learning objectives for this presentation include: (1) attendee appreciation of the linguistic complexities of applying biological information obtained from the human skeleton to medicolegal statements regarding racial identity; (2) a discussion of the strengths and limitations of current terminology utilized in scientific publications; and (3) a call for further discussion regarding the language used to describe ancestral affiliations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating that the presence and acceptance of the overlap between biological ancestry and social race should be used as a way to open discussion about appropriate usage of terminology to reach a more holistic way of defining an individual's ancestry and racial identity based on their skeletal traits.

Forensic anthropologists must often navigate the borders between a holistic, theoretical science and the practical applications of the United States medicolegal system. On the one hand, forensic anthropologists recognize that humans display normal human variation that is not categorical in nature; yet, on the other hand, they provide medicolegal authorities with ancestral assessments to aid in the identification of unidentified human remains. To do so, forensic anthropologists rely on correlations between the expressions of certain biological traits and an individual's social race. While forensic anthropologists should be cognizant of the differences between assessed skeletal ancestry and social race, the scientific literature suggests otherwise, as the terminology for ancestry and the terminology for social race are often used interchangeably in the context of human identification and human identification research.

As the field of forensic anthropology continues to grow and populations continue to change, an emphasis on the use of terminology used to define an individual's ancestry and race becomes increasingly important to discuss. A review of forensic anthropology articles in the *Journal of Forensic Sciences* and the *American Journal of Physical Anthropology* from 2008 to 2019 demonstrates a notable shift in how ancestry is defined and discussed. This may reflect larger trends in terminology and thinking in the field of anthropology as articulated by the American Anthropological Association statement on race.<sup>1</sup> Some authors have actively made the decision to emphasize that the specific terminology used in their publication may not be encompassing of all individuals classified within said group. While this emphasis in terminology is more commonly geared toward minority groups (such as Hispanics and Blacks), this idea can and should be applied to all ancestral groups of focus in the field. If the terminology used to describe an ancestral group cannot fully embrace the variation that exists within the group, a push toward a change in terminology should be the next step.

Anthropologists recognize that social race is complex, personal, and political. Colleagues in the American Association of Physical Anthropologists have recently articulated concerns regarding the overly simplistic equating of skeletal biology and social race and the potential sociopolitical consequences.<sup>2</sup> This presentation serves as an avenue to present questions and concepts to consider, such as: Why do researchers continue to narrow the definition of Hispanic and Black into distinct smaller and smaller subpopulations (for example: Caribbean Black, South Carolina Slave, Mexican, or North American Hispanic) while the definition of White seems to encompass individuals from a variety of geographic, cultural, and linguistic backgrounds? Why are these distinctions never addressed or discussed in forensic anthropology reports aiding in the identification of human remains? Should the Anthropology Section of the American Academy of Forensic Sciences consider standardizing racial terminology used in forensic anthropology reports? What is the obligation of the forensic anthropologist to consider the broader cultural ramifications associated with the use of certain racial terminologies in their reports in regard to identification and investigation efforts?

The data in this presentation collected from the scientific literature supports the hypothesis that social race is culturally defined and evolves over time. Therefore, forensic anthropologists should consider not only how racial terminology impacts identification, but also the ways in which skeletal variation and social race are understood—or misunderstood—by the larger medicolegal community. Not only is this type of discussion important to the field of forensic anthropology in relation to biological profile construction, but its importance also expands to the medicolegal system, as improved terminology could ultimately lead to an improved identification process for unidentified human remains.

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### Forensic Anthropology, Biological Ancestry, Social Race





## A14 The Use of Eye Tracking Technology in Forensic Anthropology: An Empirical Approach to Advancing the Understanding of Complex Visual Tasks in Cranial Macromorphoscopic Trait Evaluations

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**Learning Overview:** After attending this presentation, attendees will gain insights into the successful usability of eye tracking technology in forensic anthropology, method development in ancestry assessments specifically, and visual skeletal assessments more broadly.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how eye tracking approaches have the potential to be utilized to measure empirically the extent of visual attention used in complex tasks. The goal of this presentation is to demonstrate to attendees the usability of eye tracking technology as a research tool in forensic anthropology to study empirically visual gaze patterns involved in the interpretation of skeletal remains.

This presentation provides insight into the application of eye tracking technology in forensic anthropology by first, providing an overview of key research that has been conducted within this area in forensic science and, second, by presenting specific eye tracking data collected from a preliminary study in visual Macromorphoscopic (MMS) trait analyses for ancestry estimations.

The use of eye trackers as a research tool has increased in recent years, with a growing interest from a number of different disciplines.<sup>1</sup> Eye trackers are measurement devices used to capture eye movements and have been applied to assess tacit knowledge from human experts and their performance within multiple domains.<sup>2</sup> In forensic science, however, the use of eye tracking technology as a tool to study forensic decision-making is largely underutilized, with only a few published studies conducted to date.<sup>3-5</sup> Forensic anthropologists rely heavily on well-established visual methods when conducting the task of creating a biological profile. To some extent, this practice takes full advantage of the human perceptual system.

This presentation will showcase the results from an initial empirical study conducted using eye tracking technology to consider the MMS procedures used in ancestry estimation. This study sought to assess the capabilities of using eye trackers as a tool in studying visual processing and strategies involved in MMS procedures by looking at participants' *fixation points* (what features participants focus on), *fixation duration* (total time participants spend looking at the different features involved in the method), *scan path* (the order in which participants look at the features), and *visit count and duration* (the number of total visits and duration to particular areas).

The results of this preliminary study identified differences in gaze “strategies” with regard to fixation points, visit duration, and visit counts between the participants. The data generated provide a starting point for assessing how such technologies could be used in order to understand the decision-making processes involved in forensic anthropological interpretations and their role in forensic reconstructions more fully. These results will be discussed from an “applied” point of view, in terms of presenting the type of data collected, and how the data was quantified and analyzed.

Ultimately, the findings provide valuable insights into how to interpret and use the data in order to advance our understanding of the decision-making processes involved in the assessment of skeletal remains, and provide more transparent and reproducible evaluative reconstructions.

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### Forensic Anthropology, Eye Tracking, Visual Attention



### A15 Eye Tracking to Assess Decision-Making in Cranial Macromorphoscopic (MMS) Trait Evaluation: Implications of Education and Training in Method Application

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**Learning Overview:** After attending this presentation, attendees will better understand the impacts of experience and education on decision-making processes and how eye gaze behavior is associated with MMS trait analyses, method development, and method improvement.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how eye tracking technology can grant further insights into the application of forensic anthropological methods with a focus on ancestry estimation. Research in multiple forensic science disciplines delve into how technology can be used to understand decisions made by experts in their respective fields. Investigating anthropological methods from this cross-disciplinary approach can help researchers understand human decision-making, specifically how and when variance occur in method application and thus support the improvement of method development and training.

In this study, ten individuals with various education and experience levels assessed two skulls using the MMS scoring procedure while wearing Tobii Pro eye tracking glasses. Data from the glasses allowed the creation and analysis of eye fixation patterns. This includes time recorded for each of the 17 MMS traits, time spent on each individual skull, and the overall time of analysis. Individuals were assigned distinct groups based on a preliminary questionnaire. These groups are separated into the following categories: (1) education level, (2) MMS experience, and (3) a combination of both education and experience.

For this study, time was used as a proxy for confidence in decision making for the MMS method. A Kruskal-Wallis test was used to compare the times between the three groups. Kruskal-Wallis and pairwise Wilcoxon rank sum tests were applied comparing the times for each individual MMS trait between the groups. A two-way mixed-effects model for Intraclass Correlations Coefficients (ICC) was used to measure inter-observer reliability of the MMS scores on each skull.

When looking at the median times for each trait, the Nasal Aperture Width (NAW) was the quickest scored trait, with an overall median time of 14.59 seconds. The results also show that Nasal Bone Contour (NBC) took the longest to score, nearly 16 seconds slower than the next longest trait to score, the Transverse Palatine Suture (TPS).

When analyzing the median overall time to score both skulls, a Kruskal-Wallis rank sum test indicates individuals with <2 years of MMS experience and individuals with >2 years of MMS experience differed significantly ( $p=0.01052$ ). The results from the Kruskal-Wallis and Wilcoxon rank sum tests for individual MMS traits show that time differences between experience, education, or a combination of both are not significant. While none of these differences are significant, individuals with more than two years of experience were consistently quicker than individuals with less than two years of experience, with the exception of: (1) nasal overgrowth, (2) orbital shape, and (3) postbregmatic depression.

The results of inter-observer error using the single fixed rater ICC indicate reliability between the ten individuals is moderate ( $ICC=0.72-0.74$ ). When grouped by education level, the ICC results indicate good agreement between groups ( $ICC=0.86$ ). ICC results based on the experienced groups show good to excellent agreement ( $ICC=0.86-0.92$ ). Overall, when splitting the assessors into the four groups based on experience and education level, the ICC results indicate good agreement ( $ICC=0.82-0.88$ ), demonstrating that experience is the key to higher reliability between assessors. The ICC results indicate that the inter-observer reliability is high for the MMS method, with experience only slightly improving the agreement between groups.

Using the time data, ICC results, and heat maps created from the eye gaze patterns, eye tracking technologies allow researchers to visualize where variance may be occurring between assessors to identify critical parts of decision-making process. Through empirical decision-making studies, forensic anthropologists can move forward and improve practice by decreasing observer differences by targeting confusing or problematic aspects of a method. This allows training to focus on problematic traits when training forensic anthropologists.

#### Forensic Anthropology, Macromorphoscopic, Decision-Making



### A16 Metric and Non-Metric Ancestry Evaluation Analysis of the Craniofacial Region in Greek-Cypriots: A Pilot Study

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the need to create population-based standards for less-studied regions, such as Cyprus and the wider eastern Mediterranean. This study combines different ancestry evaluation techniques developed around the world in order to determine an ancestry profile for Greek-Cypriots. This study also discusses the value of the Greek-Cypriot population as a platform for the development of human identification standards that could server the rest of the eastern Mediterranean region.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a new composite method in ancestry evaluation that could serve as a population-based standard for human identification for Greek-Cypriots. This research utilizes a combination of metric analysis and non-metric observations to determine the skeletal “face” of Greek-Cypriots. This pilot study evaluates the relationship between different bones of the facial skeleton and their bordering landmarks. Thus, this study offers a unique insight of how different methods could work together to the development of a single ancestry evaluation approach for a newly studied population. Furthermore, the genetic makeup of Greek-Cypriots resembles that of its immediate neighbors; thus the results of this study, with little or no modification, could be applicable in establishing positive identification for unidentified remains retrieved in those areas.

Ancestry is a topic less studied in Europe and the Mediterranean region than in the United States. Little effort has been made to define the morphological parameters of the different populations that constitute the continent. The underlying hypothesis that we are all European-White in Europe has played a major role in ancestry being a less common research subject. However, open borders within the Eurozone and immigrant influx from war-afflicted countries have increased the need for ancestry standards to be developed as Europe and the Mediterranean are now characterized by mixed and admixed communities.

Combining both metrics and non-metric traits, the aim of this study is to create an objective methodological framework for analysis for this particular region of the cranium. The objective of this study is to define the craniofacial character of Greek-Cypriots and establish ancestry-related parameters. Methods focusing on more area-specific regions of the cranium can further aid in the evaluation of ancestry of fragmentary remains. In a pilot study of contemporary skeletons from the Cyprus Reference Research Collection (CRRC), focus was placed on the identification of macromorphoscopic and metric characteristics of the zygomatic bone in relation to the maxilla, orbits, and nasals.

A total of 50 crania were assessed using a total of 17 measurements, 5 of which aimed at recording size differences between males and females. The presence, frequency, and expression of ten non-metric traits from the zygomatic region was assessed macroscopically. While metrics show that a degree of sexual dimorphism in the overall size of this region of the facial skeleton is present, in some instances the overlap between male and female ranges was significant. Furthermore, when compared to other population groups, the mean average of bizygomatic breadth in Greek-Cypriots show distinct ancestral character specific to this population. With regard to non-metric observations, in many instances zygomatic traits classified within the low expression categories. However, some traits proved to be population specific. For example, a projecting zygoma was a predominant male (64%) and female (80%) characteristic, with an overall 72% incidence value; while the malar tubercle was greatly absent in both sexes. Additionally, the shape of the zygomatico-temporal suture was assessed, and a potentially a new non-metric trait was discovered during this research project.

In conclusion, the results of this study suggest that the combination of both metric and non-metric analysis is a useful tool in ancestry evaluation. The two approaches are complementary to each other and provide an objective framework for the determination of ancestry in skeletal remains but also reflect on the underlying similarities and differences between the sexes.

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#### Ancestry Evaluation, Macromorphoscopic, Sexual Dimorphism



## A17 Craniofacial Modularity and Integration: Implications for Ancestry Assessment

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**Learning Overview:** The goal of this presentation is to evaluate the covariance of select craniofacial features to assess integration and modularity of the facial skeleton. After attending this presentation, attendees will better understand the morphological relationships between select facial features commonly used in ancestry assessment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an understanding of the degree of craniofacial morphological covariance, identifying boundaries of correlated traits, and recognizing the implications integration and modularity may have for interpreting skeletal ancestry.

Integration and modularity describe covariation of traits and are conceptually linked. Integration refers to the coordinated joint variation of traits. When such covariation of traits is tightly correlated and internally concentrated within a region, but remains distinct and relatively independent from other traits, this is recognized as modularity. While integration and modularity of the human cranium are well acknowledged in evolutionary and morphometric studies, researching these patterns of trait interactions remains valuable as they directly affect phenotypic trait expression. Quantification of these interactions is relevant to forensic anthropologists because the form and spatial relationships of craniofacial features are commonly analyzed to estimate an unknown individual's ancestral affiliation.

Three-Dimensional (3D) coordinate data were collected from 27 identified individuals analyzed at the University of Florida C.A. Pound Human Identification Laboratory. Selection of individuals included the following criteria: (1) 3D surface scans collected using a NextEngine™ 3D Laser Scanner were available; (2) the individual's age-at-death was  $\geq 18$  years; and (3) select craniofacial landmarks were not affected/obscured by pathological conditions, trauma, or taphonomic modification. Coordinate data of 16 standard craniofacial landmarks were collected for each individual and partitioned into "upper," "middle," and "lower" non-overlapping compartments (modules) of the face. Four possible scenarios of these subdivisions were organized, each demonstrating different boundaries defining facial compartments and thus including different combinations of landmarks within compartments.

The data were analyzed in R™ using a simultaneous-fit approach, in which a generalized Procrustes fit was performed on all coordinates, followed by a series of covariance tests to assess integration and modularity ( $\alpha=0.05$ ). Two-block Partial Least Squares (PLS) analyses of the Procrustes coordinates indicated that of the four compartment scenarios, modules of the face are best compartmentalized by visceral function rather than by the mere proximity of neighboring landmarks (i.e., this scenario showed the highest degree of independence between compartments). Accordingly, skeletal landmarks on the boundaries of the orbital, nasal, and oral cavities correspond to the upper, middle, and lower compartments, respectively. Of this visceral compartmentalization scenario, pairwise PLS analyses of Procrustes coordinates between each module yielded significant results for comparisons of the orbital:nasal ( $r_{PLS}=0.78$ ) and nasal:oral ( $r_{PLS}=0.89$ ) compartments; the comparison of the orbital:oral compartments approached significance ( $r_{PLS}=0.71; p=0.06$ ). The average degree of integration between the three visceral modules was not significant ( $r_{PLS}=0.80; p=0.77$ ), suggesting that integration among these modules occurs in a chain-link pattern, with those compartments that are more closely linked spatially and developmentally showing greater magnitudes of integration. Pairwise covariance ratio tests were conducted to quantify the degree of modularity of the facial compartments and yielded insignificant results in all comparisons. These results demonstrate that despite regions of trait correlations defined by visceral functions, covariation in facial traits crosses the borders delimiting the distinct organs of special sense.

The points selected for this study, while considered standard craniofacial landmarks, correspond directly to facial features that are regularly used in assessments of skeletal ancestry. Investigating the covariation between these traits can elucidate underlying processes that produce phenotypic variation, and in this context, aid the understanding of craniofacial traits and which are biologically meaningful for ancestry assessments. In summary, this study shows that the facial skeleton can be partitioned into three modules based on visceral function that show large magnitudes of integration with one another. Importantly, this integration is not uniform throughout the face, meaning that some traits are more highly correlated with each other to the exclusion of other traits. These findings have implications for ancestry assessment, wherein methods rely on relative relationships between traits, or assessing multiple traits that this study shows to be highly integrated and are consequently not independent of one another. Therefore, this research can serve as a foundation to advance approaches to ancestry assessment.

### Trait Covariation, Geometric Morphometrics, Morphological Variation



## A18 Re-Evaluating Skeletal Ancestry Traits Using Three-Dimensional Technology

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**Learning Overview:** After attending this presentation, attendees will better understand the use of Three-Dimensional (3D) technology to quantify morphological ancestry traits on the human skull.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating possible advancements in the field of forensic anthropology, more specifically the development of a new, more precise, and standardized method of ancestry assessment using modern-day technology. With regard to competence and performance, this method can contribute to making ancestry assessments less dependent on an anthropologist's experience with human variation and more accessible to younger forensic anthropologists.

According to the Royal Canadian Mounted Police (RCMP), women of Canadian Indigenous descent, previously referred to as Aboriginal, are at a higher risk of being victims of violence than other women in Canada.<sup>1</sup> As of 2015, 204 cases of missing and murdered Indigenous women remained unsolved, making it a major concern for Canadian Indigenous communities, who are still pressing for the identification of these women.<sup>2</sup> Forensic anthropology can contribute to the identification of victims by establishing a biological profile of the skeleton, including the evaluation of sex, ancestry, and age from human remains.<sup>3</sup> Ancestry assessment describes population affinity based on two criteria: morphological analyses, which examine size and shape of bone features, and metric analyses, which use skeletal measurements.<sup>4</sup> Morphological analyses are difficult to reproduce and standardize, as they strongly depend on subjective judgments by anthropologists, based on their experience with human variation.<sup>5</sup>

The purpose of this research was to improve the rigor of morphological analyses of ancestry by using 3D technology to quantify relevant features on the human skull. More specifically, circumference/perimeter, surface area, and proportion ratios were used to characterize the 3D outline of the orbits, the nasal cavity, and the palate. The sample was comprised of 24 Canadian Inuit, 50 European-American, and 13 African-American adult female crania, for a total sample size of 87 individuals. The samples were imaged using photogrammetry, the 3D models were constructed in 3DF Zephyr, and the shape analysis was performed in 3DS Max. The data were statistically analyzed using a non-Parametric Multivariate Analysis Of Variance (PERMANOVA), a Linear Discriminant Analysis (LDA), and a Principal Component Analysis (PCA). Results showed that individually, the 3D measurements were not very informative, since there was significant overlap between groups for the different traits evaluated. However, major differences between groups were clearest when 3D measurements were combined using multivariate statistics. Overall, European-Americans were statistically different from the other two groups, while Canadian Inuit and African American individuals were harder to distinguish statistically. The current method presented a classification rate of 87.36% (jackknifed: 80.46%) and an average repeatability of 97% when all measurements were combined. Nevertheless, this project had limitations and further research should evaluate the current method with a larger sample size, more populations, other ancestry-related cranial traits, such as the oval window, and other 3D measurements, such as volume.<sup>6</sup> Despite its limitations, this new and simple method of 3D shape analysis has potential and shows promise for the future of ancestry assessment via 3D imaging.

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### Forensic Anthropology, Ancestry Assessment, Canadian Indigenous



## A19 A Comparison of Two Data Collection Methods for the (hu)MANid Program on a Diverse Sample of Mandibles

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**Learning Overview:** After attending this presentation, attendees will better understand the differences in performance of 3D scans versus direct measurements in the (hu)MANid program to estimate sex using the mandible.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the different sex classification results that are produced for two methods of data collection, further helping to improve validation and the usage of (hu)MANid.

The (hu)MANid program is a free, web-based software developed by Berg and Kenyhercz that uses mandibular metric and morphoscopic variables to assess sex and ancestry through Linear Discriminant Analysis (LDA) or Mixture Discriminate Analysis (MDA).<sup>1</sup> The program's reference database contains 1,745 individuals from 15 different populations (including modern, historic, and prehistoric groups). Berg and Kenyhercz report that MDA renders the highest accuracies among the analytical methods offered by the software, with pooled sex estimation accuracies as high as 83.5%.<sup>1</sup> A relative limitation of (hu)MANid program is the use of some mandibulometer measurements, due to: (1) the error rates in some of them, and (2) the cost of the instrument.<sup>2,3</sup> 3D surface scans (3D scans) may provide an alternative method for data collection. The aim of this study was comparing the (hu)MANid accuracies of 3D scans and direct measurements of a diverse sample.

Six metric and morphoscopic mandibular measurements were collected from 3D scans of mandibles from recent United States Black, United States White and Portuguese, as well as medieval Nubian and prehistoric Native American of both sexes ( $N_{total}=555$ ). The same nine metric and morphoscopic measurements (including three mandibulometer measurements) were collected by direct measurement of mandibles from modern males and females from the Maxwell Museum Donated Body Collection ( $N_{total}=59$ ). These data were run through (hu)MANid for pooled sex classification. MDA was applied due to its higher reported correct classification and its consideration of both metric and nonmetric measurements.<sup>1</sup>

For both samples, no significant differences were detected between non-stepwise and stepwise options; therefore, non-stepwise option was employed in the remaining analysis. Overall, the direct measurements rendered average correct classifications similar to those originally reported for the program (85% vs. 83.5%, respectively), while those of the digital measurements were lower (65%). Males displayed similar correct classifications for the digital (93%), direct (92%), and direct-with-mandibulometer (93%) measurements; however, they differed in positive predictive power (60%, 90%, and 87%, respectively). Females varied significantly in their correct classifications for the digital (36%), direct (55%), and direct-with-mandibulometer (36%) measurements; furthermore, they differed in positive predictive power (82%, 60%, and 67%, respectively).

For pooled sex estimations, the (hu)MANid program is better than chance, but males are more likely to classify correctly with the direct measurements than females. Despite the smaller sample size, the direct measurements had higher correct classification, but the mandibulometer measurements did not increase the overall correct classification. However, further evaluation using more variables is suggested.

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### (hu)MANid Program, Sex Estimation, Mandible



## A20 Craniometric Variation and FORDISC® Misclassification in Latin American and Asian Individuals

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**Learning Overview:** After attending this presentation attendees will better understand the impact of craniometric variation on the estimation of ancestry using a unique combination of Latin American and Asian samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the limitations of estimating ancestry for Latin American individuals using craniometrics in regions with population histories derived from both Latin American and Asian populations. This presentation will also demonstrate the need to include additional samples from these populations in FORDISC®.

This study explores cranial morphology and the usage of traditional morphometric ancestry estimation software to assist in the identification of deceased individuals in a Latin American sample. Recently, the complexity of morphological variation in Latin Americans has become increasingly discussed due to the changing demographics of the United States and the challenges admixture poses on identification cases.<sup>1</sup> Although the term Hispanic is traditionally used to classify individuals of Latin American descent, this classification fails to accurately describe populations in cultural and biological contexts, and this misrepresentation extends to modern methods of ancestry estimation.<sup>2</sup> In some areas of the United States, such as California, there are long histories of residency by both Latin Americans and Asians, which necessitates an ability to distinguish ancestry between the populations for cases in which context and visual examination do not point to a particular population. FORDISC 3.1® (FD3), a discriminant function analysis-based software, has been shown to misclassify Latin Americans as other ancestries, such as Japanese.<sup>1</sup>

In this study, FD3 was run on a sample ( $N=360$ ) of Latin Americans (from the Hanihara dataset and Latin American Craniometric Database) comprised of individuals from Mexico, Colombia, Peru, Chile, Venezuela, Bolivia, and Argentina, along with individuals of Asian origins ( $N=470$ , including Japan, China, Philippines, and Java from the Hanihara dataset) to ascertain its accuracy in distinguishing between these populations. Analyses were separated by sex and include Mahalanobis  $D^2$  (a biodistance measure), Principal Coordinates Analysis (PCoA), Canonical Variates Analysis (CVA), the Neighbor-Joining (NJ) method for construction of phylogenetic trees, and  $F_{st}$ . The hypothesis tested is that FD3 will be limited in classifying Latin Americans in the presence of selected Asian reference samples.

The results of these analyses show FD3 misclassifications exceeded 50% for Latin Americans as a group, and by individual sample, which is less than chance. The PCoA, which reflects the biodistance matrix, shows similarities between Asian and Latin American populations, and large differences among Latin American populations, such as Mexico and Peru. Further, CVA shows Asian populations spread across CV 1 and CV 2 in males, while Latin American samples were primarily constricted to positive values of CV 1 and the center of CV 2. Females yield similar CVA results where Asian and Latin American populations overlap one another with little clustering by sample. NJ analyses yield results consistent with CVA.  $F_{st}$  values (males=0.1152, females=0.1287) were high in comparison to populations around the globe.

FD3 as a tool is not currently helpful in scenarios in which context and visual assessment cannot narrow potential ancestries because the selection of reference groups may include both Latin American and Asian samples. For practitioners in areas such as California with a long and rich history of American Indian, Latin American, and Asian residents, among many others, these classification percentages leave much to be desired. These results join other studies that call for the need of representative samples, which can be applied to diverse populations and facilitate the work of forensic practitioners so that victims of homicide, genocide, exposure, and suicide can be identified and returned to their families. This is particularly critical for border crossers in the United States southwestern border.

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### Cranial Variation, Admixture, Misclassification



## A21 Geographic Origin Estimation of Latin American Individuals Using Craniometric Data

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**Learning Overview:** After attending this presentation, attendees will understand how geographic origin estimation in forensic anthropology can provide a finer-grained approach than ancestry identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by further exploring the biological variation found in Central America and Mexico to help improve biological profile methods for unidentified migrants.

The lack of information surrounding the term “Hispanic” creates difficulties when attempting to estimate geographic origin due to the variation encompassed by this term. Exploring the biological variation between Mexican and Guatemalan populations allows for investigation of geographic origin rather than the classification systems currently utilized in forensic anthropology. If geographic origin can be estimated based on craniometric measurements, it could potentially narrow a missing person’s list. This could also be used to help facilitate and potentially expedite the identification and repatriation of unidentified migrants. The purpose of this presentation is to use positively identified and contextually documented individuals from Mexico and Guatemala to explore the variation within and among these two geographic regions, as well as sub-regions, to provide a foundation for improving ancestry estimation. Moreover, this research attempts to address the following questions: (1) Can a reference sample of Guatemalans, used as a proxy for Central America, be distinguished from a Mexican population via cranial morphology? (2) Can sub-regions (Central, North/West, and Southeast) of Mexico and Guatemala be distinguished from each other via cranial morphology?

To answer these questions, this research utilizes individuals from four institutions, including the Pima County Office of the Medical Examiner, the Forensic Anthropology Foundation of Guatemala, Operation Identification at Texas State University, and the School of Anthropological Sciences of the Autonomous University of Yucatán, at Mérida. Together, this sample includes individuals migrating to the United States from Guatemala and Mexico and indigenous Guatemalan Mayans. Howells inter-landmark distances were collected from each cranium using a MicroScribe® digitizer and the program 3Skull.<sup>1,2</sup> A discriminant function analysis and canonical variates analysis were performed to assess the variation and classify the individuals. The Guatemalan sample can be correctly classified 75.25% of the time when compared to Mexicans, and the cross-validation rate suggests that when the three Mexican regions are compared (Central, Southeast, and North/West), they can be differentiated 77.8% of the time. The Mahalanobis Distance matrix from the regional comparison indicates the Southeast Mexico group is statistically different from the Central and North/West groups ( $p < 0.001$ ). When Guatemalan Mayan individuals are included in the Mexico regional comparison, the cross-validation rate decreased, but the Mahalanobis Distance matrix showed that the Southeast group and the Guatemalans were statistically different from all groups. With Guatemala used as a proxy for Central America, complex craniometric patterns have begun to emerge that need further exploration. These results suggest that other regions of Central America are also distinguishable from Mexico. This research indicates that even though the three regional Mexican groups can be separated, the large difference in cranial variation is between the Southeast region and the remainder of Mexico. Considering these patterns, this research indicates geographic origin estimation can be used in lieu of broad ancestry estimation in forensic casework. This could potentially eliminate broad terms such as Hispanic, which are less informative in a forensic setting, particularly when considering migrant remains.

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### Latin America, Craniometrics, Biodistance





## A22 The Impact of Error in Femur Subtrochanteric Measurements on the Assessment of Population Affinity

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**Learning Overview:** After attending this presentation, attendees will understand the implications of intra- and inter-observer measurement variability on the calculation of Platymeric Index (PI) and resulting ancestry classifications. This presentation focuses on the reliability of the required measurements and the consistency of index classifications.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the effect of measurement error on a commonly utilized method for assessing population affinity from postcranial skeletal remains.

When skeletonized remains are incomplete and/or fragmentary, the PI has been identified as a possible method for population differentiation.<sup>1,2</sup> This method serves as a part of the forensic toolkit for the estimation of ancestry and is most commonly utilized when cranial elements are missing or fragmented. However, previous research on data accuracy and reliability indicates that measurements utilized to calculate the PI, the subtrochanteric Anterior-Posterior Diameter (APD), and Medial-Lateral Diameters (MLD) of the femur have greater-than-acceptable error rates.<sup>3</sup> Despite reported error rates exceeding that of the published thresholds, the measurements remain necessary for assessing population affinity in forensic casework. Thus, it is important to understand the role of intra- and inter-observer error in the data collection process and how that variability may impact method application and classification results.

In this study, three observers independently collected three rounds of APD and MLD measurements on 53 left and right femora. The femora are part of a commingled assemblage of Vietnamese males derived from a C-130 aircraft that crashed in 1974. In an effort to emphasize replicability of the measurements across time and ensure that each round was performed independently with no relation to past rounds, a minimum of one week passed between each round.

Absolute and relative Technical Errors of Measurement (TEM) were calculated to assess measurement error within and between observers. APD and MLD measurements were then utilized to calculate the PI (APD/MLD x 100). Indices were classified as platymeric, eurymeric, and stenomic following sectioning points recommended in previous studies to demonstrate the role of inter-observer measurement variability on classifications, and as a result, the potential effect in ancestry estimation application.<sup>1,2</sup> Comprehensive Kruskal-Wallis and chi-square tests of significance were conducted to determine the degree of intra- and inter-observer error throughout the analyses.

Results mirror previously reported observer errors with absolute TEM ranging from 0.50mm to 0.77mm and relative TEMs ranging from 2.10% to 3.17% for intra-observer error. Inter-observer absolute TEM ranges from 0.88mm to 1.14mm and relative ranges from 2.98% to 4.65%. Despite greater-than-acceptable error in the raw measurements, no significant differences were found between measurement rounds or index calculations. Likewise, no significant differences were observed in the classification data for each observer.

These results highlight both the reliability and validity of subtrochanteric femur measurements when applied to PI classifications. While intra- and inter-observer error remains high in the raw measurements, it is not enough to significantly alter the platymeric classification results for this dataset. Thus, the present analysis argues that variation in how the PI of individuals or groups classify is not likely a result of measurement error, but rather a reflection of method application and the specific groups included in the given analyses.

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### Platymeric Index, Subtrochanteric Measurements, Data Validation



## A23 Odontometric Variation of Male Skeletal Samples From the United States and South Africa

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**Learning Overview:** After attending this presentation, attendees will better understand population variation in tooth size and how odontometrics can be used as part of the biological profile.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting tooth size variation across continents and its relevance to forensic anthropology.

The utility of dental crown measurements in the estimation of ancestry has proven to be a promising approach.<sup>1</sup> However, more work is needed to explore global modern human variation in terms of tooth size to better understand secular change and the role of evolutionary forces (i.e., gene flow and drift). To address this deficiency in knowledge, this study compares odontometric data from modern skeletal collections representing self-identified Black and White individuals in the United States and South Africa.

The South African samples were obtained from three collections: the University of Pretoria, the Raymond A. Dart collection, and the Kristen collection. Dental data from the United States were collected from the Bass Collection at the University of Tennessee, Knoxville, and the donated collection at Texas State University, San Marcos. Only individuals with known sex and ancestry were included; due to sample size constraints, only male individuals were included in this study ( $n=397$ ). Maximum crown dimensions and cervical crown dimensions were collected following Hillson et al.<sup>2</sup> Dental measurements were collected from the left side of the arcade using digital dental calipers. In cases of missing or damaged teeth, the right antimere was substituted. Population differences were explored using a Kruskal-Wallis test in the R statistical computing environment.

Dental measurements between South African Blacks and American Blacks differed significantly in nearly every maximum crown dimension. Differences in mesiodistal crown measurements were most pronounced, being present in all teeth except the upper and lower lateral incisors. Differences were less pronounced in measurements of the crown at the cervico-enamel junction. Overall, American Black dental dimensions were greater than those of South African Blacks. When comparing South African and American Whites, only two significant differences were present: in the buccolingual cervical measurement of the lower second molar (larger in American Whites) and in the mesiodistal cervical measurement of lower third molar (larger in South African Whites).

The results of this study suggest that American and South African Blacks differ considerably in their dental dimensions. These findings are consistent with the notion that American Blacks have been subject to considerable gene flow and may stem from a different source population in west Africa. These results are further in line with the finding that there is generally greater diversity within the African continent than the rest of the world. Conversely, the South African and United States White samples do not greatly differ and have on average smaller teeth, suggesting greater population affinity between these groups and less change over time. The impact of evolutionary forces and secular change on tooth size should be further explored and considered in ancestry estimation methods, particularly in the development of appropriate reference samples.

*Funding provided by a National Institute of Justice award.*

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### Ancestry, Gene Flow, Tooth Size



## A24 Influence of Ancestry on Sexual Dimorphism in the Human Mandibular Canine

Brittney L. Blevins, MA\*, Lexington, KY 40504

**Learning Overview:** After attending this presentation, attendees will gain a better understanding of the ancestral differences in sexual dimorphism observed in measurements taken from human mandibular canines.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an alternative method to estimate sex in skeletonized individuals.

Sexual dimorphism can be quantified using human mandibular canines, which allows these teeth to be used to estimate sex of skeletonized individuals. This study complements and expands on previous research by using the Mesiodistal Diameter (MD) and Buccolingual Diameter (BL) of human mandibular canines to assess sexual dimorphism between three ancestry groups. Measurements were taken from a sample of skeletal individuals from the Terry Collection ( $n=651$ ), the Maxwell Museum of Anthropology Skeletal Collection ( $n=61$ ), and a random sample of dental casts from the James K. Economides Orthodontic Case File (JKE) ( $n=599$ ), for a total of 641 males and 597 females. Three ancestral categories were used: American Black, White, and Hispanic. A total of 50 individuals of each ancestry were randomly selected for all reported statistical analyses.

Previous research using this data set has reported a significant difference between males and females for left and right BL measurements ( $p<0.0001$ ), indicating that males have significantly larger BL measurements of the mandibular canine than females from the study sample.<sup>1</sup> However, when individuals are separated by ancestry group (as recorded by each collection) only American Black individuals show significant levels of sexual dimorphism for the MD measurement ( $p=0.0012$ ) and BL measurement ( $p<0.0001$ ). Although race is a socially constructed concept, not a biological reality, there is a history of segregation between various groups of individuals that might explain the difference in the presence of sexual dimorphism shown between individuals of different ancestries. This study is consistent with other research in finding odontometric differences between different ancestral groups.<sup>2-4</sup> Since significant differences between males and females was only observed in American Black individuals, logistic regression equations were not created for other ancestral groups. Logistic regression equations were created to estimate sex based on measurements from either the left or right mandibular canine. These equations correctly estimated the sex of individuals in up to 73.33% of cases when using only the BL diameter measurement. Accuracy of the equations varies depending on whether the measurements are used individually (BL or MD) or combined (both BL and MD). Equations more accurately estimate the sex of male individuals than female individuals. This method may be useful for estimating the sex of skeletal individuals in forensic cases when traditional methods are not applicable but could only be used for cases whose ancestries have been estimated to be American Black. Future research will further explore the impact of secular change on sexual dimorphism and ancestral differences in the mandibular canines.

*This research was funded in part by a Robert C. West Graduate Student Field Research Award from the Department of Geography and Anthropology at Louisiana State University.*

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### Odontometrics, Sexual Dimorphism, Ancestry

## A25 Human Trafficking: A Challenging Case of Age Assessment

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**Learning Overview:** After attending this presentation, attendees will better understand the importance of careful clinical examination to uncover anomalies that could influence bone growth or dental maturation when the assessment of biological age is required.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enlightening the need of a multidisciplinary approach to age estimation. When a Victim of Human Trafficking (VHT) has to be evaluated, psychological maturity and physical appearance must be considered.

There are approximately 800,000 people trafficked across international borders annually. Due to its geographical position, Italy is one of the main countries of destination for human trafficking via the Central Mediterranean route.<sup>1</sup> According to recent estimates, the number of VHTs who arrived in Italy by sea for sexual exploitation has increased by 600% in the past three years. Nigerian girls aged between 16 and 17 are the most affected by this phenomenon, accounting for more than 90% of all victims.<sup>2</sup> The number of applications for forensic age assessment in undocumented VHTs drastically increased in the last period because of the prevalent involvement of minors close to 18 years of age. In Italy, the proof of being over or under the age limit of 18 years is required for legal decisions about procedural privileges and social benefits.<sup>3</sup>

A multidisciplinary evaluation, including a clinical, radiological, dental, pediatric, and psychosocial assessment, is recommended.<sup>4</sup> However, sometimes a discrepancy between physical and psychological maturity estimations occurs. In these cases, a comprehensive clinical examination is fundamental to disclose signs suggestive of conditions capable of interfering with the maturation rate of the individual.<sup>5</sup>

This presentation reports a challenging case of multidisciplinary age estimation of a six-month-pregnant VHT, who had been trafficked from Nigeria and sexually exploited in Italy. She claimed to be 20 years of age, but no identification document was provided.

The cognitive development resulting from the psychosocial assessment corresponded to a maturity degree consistent with adulthood, even considering the traumatic events she suffered. Skeletal radiology was not feasible due to the advanced stage of pregnancy. The absence of erupted lower third molars and their immaturity at Orthopantomogram (OPG), performed before the pregnancy, pointed to a minor age. A closer examination of the OPG images showed unusual alterations of mandibular trabecular bone and changes of tooth shape and size, particularly at the pre/molar level. Thus, further investigations were performed. Anemia with increased Red blood cell Distribution Width (RDW) and qualitatively abnormal Hemoglobin (Hb) were found. The diagnosis of Sickle Cell Trait (SCT) was reached through electrophoresis and genetic testing. SCT is a benign form of hemoglobinopathy with heterozygous inheritance affecting up to the 20%–30% of the Nigerian population. The homozygous form is known to cause impaired growth and a delay in teeth development.<sup>6</sup> Although individuals with SCT are normally asymptomatic, recent studies have demonstrated significant higher frequencies of radiological dental and bone changes, including hypercementosis of the roots, changes in tooth shape and size, and stepladder-pattern bone alteration.<sup>7</sup> In this case, OPG findings were consistent with these modifications rarely described in literature. The hematologic disease could cause a delay in teeth eruption and mineralization, leading to an underestimation of chronological age. Based on these findings, the age reported by the girl was deemed reasonable.

This presentation stresses the importance of including a careful clinical examination in age estimations to avoid ethically unacceptable mistakes. Physicians should be aware that some diseases can interfere with physical development; most of these conditions lead to growth delay, causing an underestimation of age.<sup>5</sup> Since, in Italy, being less than 18 years old ensures benefits from a wide range of protection measures, such underestimation would not disadvantage the person concerned. However, the main purpose of age assessment is to re-establish the identity of a VHT. Therefore, it is important to exclude these particular medical conditions to ensure an appropriate, reliable, and genuine social integration, consistent with the physical and psychological maturity degree of the VHT.

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### Multidisciplinary Age Estimation, Human Trafficking, Sickle Cell Trait

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## A26 Age-Old Problems: Using the Pars Basilaris to Estimate Age-At-Death in Non-Adult Individuals

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**Learning Overview:** Estimating the age at death of an individual is important within numerous anthropological, forensic, and archaeological disciplines to establish a biological profile. The pars basilaris has long been used and purported as a bone that can determine accurate age-at-death estimates for non-adult individuals. However, little is documented about why this bone should be used or the benefits of the age estimates generated from these metrics over other bones of the skeleton. Importantly, this study demonstrates to attendees how the biology of the growth and development of this bone makes the pars basilaris vital in generating accurate age-at-death estimates. Furthermore, it also establishes that the pars basilaris is more consistent with both chronological and dental age estimates than any age estimates generated by the long-bones. The goal of this presentation is to encourage practitioners to place greater value in estimating age from the pars basilaris than any other skeletal element.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the current limitations of using metric assessment of skeletal remains to estimate age. As a consequence, it will be demonstrated to attendees why and how using the pars basilaris has been found to be the most reliable skeletal element to assess to date. This is intended to influence current practitioners, encouraging them to consider age estimates derived from the pars basilaris with increased value.

The study of the growth and development of non-adults provides key insights into the age of the individual at death. Today, studies of non-adults have primarily focused on generating age-at-death estimates from dental development as this is known to be a reliable proxy for chronological age. However, the dentition is not always available, particularly in perinatal or infant skeletal remains, as the developing tooth buds and cusps are often lost or not recovered due to the larger tooth crypts. In the absence of the dentition, long-bone diaphyseal lengths are most commonly used to estimate age. This is problematic given the degree of genetic variability in diaphyseal length and because growth is strongly affected by adverse circumstances, such as malnutrition, resulting in large inaccuracies when used for estimating age at death.

Measurement of the pars basilaris has long been used to determine accurate age-at-death estimates for individuals less than 6 years of age as this is when it commences fusion to the pars lateralis of the occipital bone. However, little is documented about why the pars basilaris should be used or the benefits of the age estimates generated from these metrics. This study determined age estimates from metric assessment of the pars basilaris for individuals from both archaeological and historical collections where either chronological age is known ( $N=50$ ) or age estimates based on dental development are possible ( $N=173$ ) to test the accuracy of the pars basilaris in generating ages at death. Findings from this study support the conclusion that the pars basilaris is more closely correlated with chronological age than diaphyseal length because it is less susceptible to growth delay as a consequence of environmental adversity.

This study proposes that the pars basilaris is much more stable in its growth, providing more accurate and reliable age-at-death estimates for all individuals, regardless of their stress exposure and/or their disease/health status. Consequently, this presentation demonstrates the usefulness of the pars basilaris in estimating ages at death of non-adult individuals, particularly in lieu of the dentition being present, and encourages practitioners to use this element more widely within skeletal analyses.

### Aging, Juvenile, Infant



## A27 Why Cranial Sutures Should be Included in Adult Age-At-Death Estimates

Jessica L. Campbell, PhD\*, SNA International, Alexandria, VA 22314; Stephen P. Nawrocki, PhD, University of Indianapolis, Indianapolis, IN 46227-3697

**Learning Overview:** After attending this presentation, attendees will better understand how cranial suture fusion counterbalances the age-at-death estimate when combined with more reliable and commonly used adult age-at-death indicators.

**Impact on the Forensic Science Community:** This presentation will impact the forensic sciences community's ability to estimate age at death more accurately and precisely by minimizing overall mean error with the application of non-destructive and familiar macroscopic methods.

Cranial sutures are perhaps the least popular macroscopic age indicator for the human skeleton. With very few advocates, most scholars and practitioners only use cranial suture fusion when there are limited options to estimate age at death, and it is normally advised to use suture fusion with at minimum a second, more reliable indicator. Regardless of their vilification, cranial sutures do provide age-related information. They also persevere in research and practice because they are necessary in the event that other age indicators are absent or too damaged to provide reliable age estimates.

Research on the cranial sutures to date has focused on testing established methods or on evaluating which suture sites provide the most reliable information. However, most prior publications fail to place cranial sutures within the broader framework of age estimation or investigate how they perform when used with other well-known and relatively accurate and precise methods. The primary objective of the current research, therefore, is to determine how the inclusion of cranial sutures with other age-estimation methods can influence the final composite age-at-death estimate. Since there are multiple statistical strategies for combining multiple age-estimation methods, this research also tests whether the mode of statistical combination alters the outcome of the primary objective.

Data were collected from the Bass Donated Collection in Tennessee ( $n=120$ ) and the University of Pretoria Bone Collection in South Africa ( $n=132$ ), for a total sample size of  $n=252$  females and males of White and Black ancestries. Nawrocki's cranial suture fusion method, which scores ectocranial, endocranial, lateral, and palatal sutures was used to score all crania. The Suchey-Brooks Pubic Symphysis (PS), Osborne et al. Auricular Surface (AS), and İşcan et al. sternal rib end (Ribs) methods were selected because they are non-destructive and regularly used to estimate age. Each skeleton was scored blind using all four methods. Target age estimates and 95% prediction intervals were obtained for all individuals using published tables and equations; a summary age was calculated by averaging the target age estimates to allow comparisons. Mean measures of error (including bias and inaccuracy) were used to evaluate whether there was improvement in the age-at-death estimation when cranial sutures were incorporated. The final step evaluated alternate statistical strategies for consolidating individual target ages into a single summary age.

Mean absolute error (inaccuracy) was lowest when the cranial sutures were combined with the pubic symphysis, auricular surface, and sternal rib ends. When all four methods were averaged together, the mean absolute error was 12.1 years and the bias dropped to a low of -5.4 years. When the cranial sutures were excluded, mean absolute error increased to 14.4 years, with a bias of -11.2 years. The inclusion of cranial sutures in the consolidated age estimate was statistically significant for both inaccuracy and bias. These results were consistent when the data were grouped by binary sex and when the data were combined using alternate statistical strategies.

The positive influence cranial suture fusion had on the PS, AS, and Ribs can be explained by the fact that the sutures were the only independent indicator to return a positive bias in the total sample. In addition, the sutures returned the lowest inaccuracy and bias in the older decades of all the methods used in this study. These results indicate the cranial sutures act as a counterweight to help balance the expected tendency of the PS, AS, and Ribs to regress to the mean. To illustrate the implications of this finding, a bias level set to within +/-10 years allowed age to be predicted from approximately 42–70 years when sutures were included, as opposed to approximately 25–59 years when sutures were excluded.

As a result of this study, it is recommended that cranial suture fusion be included with the PS, AS, and Ribs when estimating the age of individuals that may be older than 40 years.

### Cranial Sutures, Age-At-Death, Multifactorial

## A28 A Comparison of Two Dental Age Estimation Methods Using Transition Analysis (TA)

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**Learning Overview:** After attending this presentation, attendees will be familiar with a revised application of TA for age estimation in subadults. The goal of this study is to validate the application of this method on a new populational dataset using a French dental radiographic sample ( $n=1,380$ ). This study is part of a larger project examining subadult dental age estimation using TA.

**Impact on the Forensic Science Community:** This study will impact the forensic science community through evaluation of the performance of TA analysis on dental age estimation, using original and revised reference frameworks. The evaluation of the performance of TA on this sample illustrates the complexities of subadult dental age estimation.

The dataset comprises panoramic dental radiographs from the University of Bordeaux.<sup>1</sup> Individuals are from southern France (336 females; 639 males) with ages ranging from 1 to 16 years. Radiographs were obtained from public orthodontist offices and private hospitals, representing a range of variation in socioeconomic status. The data excludes any pathological or anomalies in tooth numbers.

The sample was scored following the Demirjian et al. (DGT) method in which eight mandibular teeth are assigned a value from A to H, corresponding to crown and root development.<sup>2</sup> These scores were converted to the Moorrees et al. (MFH) scoring stages (1–14) following Phillips' conversion chart.<sup>3,4</sup> This system correlated DGT and MFH stages by aligning original definitions for each stage. DGT scores that fell between two MFH stages were compared using both MFH stages (rounding up or rounding down).

The MFH dental development scores were analyzed following the TA method for age estimation based on dental development by Shackelford and colleagues.<sup>5</sup> The data were run again through a revised method, which adapted the Shackelford et al. framework to modern reference sample data.<sup>6</sup> Here, maximum likelihood age estimates and Confidence Intervals (CI) at the 50% and 90% were compared with known age.

CIs were calculated for only 1,351 individuals due to instances of single tooth observations. When rounding scores up to the nearest MFH stage, coverage for the original method was 53.89% at the 50% CI and 90.16% at the 90% CI, while the same scores in the revised method performed at 30.87% and 72.17%, respectively. When rounding down, the original method underperformed at the 50% CI (44.90%) and the 90% CI (84.68%); however, the revised method performed better at 43.30% and 79.79%.

These results suggest that the original MFH transitions, especially when rounding up a stage, are outperforming the revised method for the Bordeaux sample. When compared to an earlier study, the original TA method underestimated age by up to three years on a test sample from the United States, while the modified sample reflected more accurate estimations. The revised method was developed on a geographically diverse sample and was assumed to perform better than the original MFH reference sample. The poor coverages may be due to the direct conversion of the DGT scores, or a lack of appropriate reference data in the revised method sample. Future work will focus on re-scoring the entire Bordeaux sample directly into the MFH system and reexamining CI coverages.

*This project was supported by an award from the National Institute of Justice. This opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the Department of Justice.*

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### Dental Development, Age Estimation, Biological Profile



### A29 The Ability to Obtain Full DNA Profiles From Nail Clippings After Long-Term Storage at Room Temperature Could Impact the Process of Human Identification

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**Learning Overview:** After attending this presentation, attendees will better understand how nail material obtained from both living and deceased individuals can provide informative DNA profiles following storage at room temperature.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to a growing body of knowledge regarding the use of alternative and often neglected sources of DNA profiles utilized for positive personal identification.

Saliva and blood are commonly used in the medicolegal system to produce DNA profiles; however, these bodily fluids are no longer an option for decomposed or skeletonized human remains and must be stored in a refrigerator or freezer until the DNA purification process can take place. Bone can be utilized as a source of DNA without cold storage but, depending on the size of the skeletal element being held, can require a great deal of secure storage space. Additionally, the DNA extraction and purification processes destroy the hard tissue of the bone, which may be needed for subsequent forensic anthropological analysis. Nail clippings, if present, can serve as the source of a DNA profile when soft tissues are not present and when the bone is needed for additional forensic analysis.

Toenails collected from six deceased and ten living individuals were used to test the following null hypotheses: (1)  $H_{n1}$ —there is no relationship between DNA quantity from human nails and time stored; (2)  $H_{n2}$ —there is no relationship between DNA quality from human nails and time stored; and (3)  $H_{n3}$ —there is no difference in DNA quantity and quality between nails taken from living and deceased individuals.

DNA was extracted from the nails using the QIAGEN® QIAamp® DNA Mini Kit with modifications to the manufacturer's instructions to allow for an extended dissolution process. The samples were quantified using Quantifiler® Trio on an Applied Biosystems® 7500 Real-Time PCR System, amplified with the GlobalFiler™ PCR Amplification Kit, and analyzed on an AB SCIEX™ 3130xl genetic analyzer. The nails collected from living individuals were sampled for extraction three times during the two-year time period: after 1 month, 14 months, and 24 months of storage. The nails collected from deceased individuals were sampled for extraction at the beginning and end of ten months of storage.

This study failed to reject all three null hypotheses. Although the means for DNA quantity decrease over time, repeated measures of Analysis of Variance (ANOVA) indicates that these decreases are not statistically significant for either living or deceased individuals. All samples obtained from both groups produced full, single-source DNA profiles at each time of analysis. The samples obtained from living individuals were compared to reference profiles obtained from buccal swabs. All profiles remained consistent with the references at each time of analysis. Thus, there was no recognizable relationship between DNA quality and time stored. There was not a statistically significant difference ( $p < 0.05$ ) between the quantity of DNA obtained from the nails of living or deceased individuals. All samples produced full DNA profiles at each time variable. Therefore, there is no significant difference between the ability to generate a full DNA profile from nails taken from living versus deceased individuals.

These results indicate that nail clippings can be utilized for DNA analysis and serve as a viable alternative to more readily used tissues, such as blood and bone. However, the sample sizes were small, so this study should be considered a pilot study only. The use of these keratinized cells for genetic analyses could impact the way biological material is archived for identification purposes by: (1) reducing the need for cold storage, (2) decreasing the amount of necessary secure storage space, and (3) preserving the skeletal material for forensic anthropological analyses.

#### DNA Analysis, DNA Identification, Toenail Clippings





## A30 The Applicability of Intralimb Indices in the Subadult Biological Profile

*Elaine Y. Chu, MA\*, University of Nevada, Reno, Reno, NV 89557; Kyra E. Stull, PhD, University of Nevada, Reno, Reno, NV 89557*

**Learning Overview:** After attending this presentation, attendees will understand how differences in skeletal growth through the lens of brachial and crural indices can assist in estimating aspects of the biological profile for subadult skeletal remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new perspective for estimating sex, age, and ancestry at country and subpopulation levels.

Body proportions are often used in areas of biological anthropology that explore modern human origins and human variation. Brachial and crural indices, comparisons of the upper and lower segments of the upper and lower limbs, respectively, have primarily been used to assess latitudinal differences in body size following Allen's and Bergmann's rules. The utility of intra-limb indices have yet to be explored as a tool for forensic skeletal estimation, especially in situations in which limited skeletal elements are recovered. The proximate aim of this study is to explore whether brachial and crural indices can be used to develop methods to estimate age, sex, and ancestry in subadults to facilitate positive identification. The ultimate aim of this study is to quantify the ontogenetic appearance of proportions; currently, all studies have looked at subadults as a homogeneous group rather than a heterogeneous group that experiences an exorbitant amount of skeletal change.

Diaphyseal dimensions were collected from roughly 900 contemporary children between the ages of birth to 20 years from the United States and South Africa. Brachial and crural indices were subsequently calculated using diaphyseal dimensions. Children were represented by four different ancestry groups with largely comparable age distributions: American Indian, Hispanic, White, and Black. Individuals were classified into one of four life history stages: Infant (birth to 3 years), Child (3 to 7 years), Juvenile (7 to 12 years), or Adolescent (12 to 16 years). Long bone lengths were also collected on individuals between 16 and 20 years of age to represent an older sample that is comparable to adults. Kruskal-Wallis tests were conducted to test for differences among mean brachial and crural indices by sex, life history stage, ancestry, and country. When showing statistical significance, a post-hoc Dunn's test was used for pairwise comparisons for a more in-depth analysis. Subsequently, Linear Discriminant Analysis (LDA) was then performed; the sample size was large enough that a training sample with k-fold cross-validation was used to create classification models for each area of the biological profile, then a test sample was used to generate realistic accuracy rates.

Results of the Kruskal-Wallis test revealed statistically significant differences between mean brachial and crural indices based on the life history stage, ancestry, and country. The LDA models produced accuracy rates ranging from 40%–90%, with brachial and crural indices showing the highest prediction accuracy for country, even in the younger ages, and population having the lowest accuracies.

Results demonstrate that proportional differences exist at a country level in early ages and this is in accordance with previous studies that use interlimb indices to evaluate Allen's and Bergmann's rules. While not all accuracy rates are at practically applicable levels, increased sample sizes and more uniform distribution of sub-groups may increase accuracy rates in future analyses. This study provides evidence for the importance of exploring the utility of methods for measuring body size variation that is not commonly used in forensic anthropology.

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### **Intralimb Indices, Growth Patterns, Body Size Variation**



### A31 The Application of Non-Destructive Dental Age Estimation (DAE) Methods Using Root Translucency on Latin American Hispanics

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**Learning Overview:** After attending this presentation, attendees will recognize DAE methods based on root translucency are efficiently accurate age estimating methods in that they are non-destructive and require minimal training or equipment. Attendees will be aware of current events, such as the humanitarian crisis along the United States' southern border, and be inspired to use their anthropological skills to make an impact and change locally and globally.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by drawing attention to underutilized aging techniques that apply to a commonly used skeletal element: dentition. Additionally, this presentation will demonstrate the versatility and applicability of said methods to the underresearched Central American Hispanic population. The age data generated from this research for an unidentified migrant population will be available for future investigations.

A humanitarian crisis is occurring along the United States-Mexico border as the number of apprehended and deceased migrants increases. Rarely are any forms of identification found with the migrant remains. Operation Identification (OpID) is a humanitarian effort created to improve the identification and repatriation process of deceased migrants by means of forensic anthropological methods. Very few of these methods have been validated on a Central American Hispanic population.

The purpose of this presentation is to explore the application of four non-destructive DAE methods proposed by Bang and Ramm, Lamendin et al., González-Colmenares et al., and Ubelaker and Parra on a representative adult Central American Hispanic population to aid this effort.<sup>1-4</sup> Age was estimated for 40 OpID forensic cases and Mean Absolute Error (MAE) for each method was compared. The methods performed from most to least accurate as follows: Lamendin, González-Colmenares, Ubelaker and Parra, and Bang and Ramm.<sup>1-4</sup> The age cohort comparisons followed similar patterns to previously reported findings where accuracy increased with the older age groups. DAE methods were the least accurate when compared to macroscopic and histological age estimation methods. Lamendin's method produces the most accurate age-at-death estimates; therefore, it is a recommended method for OpID forensic cases. Individuals aged 40–49 years of age are represented among the OpID forensic cases, making said DAE methods applicable for these individuals. Although individuals younger than 40 years of age are often overestimated, this research demonstrates that the age range based on MAE included the known ages for all identified individuals ( $n=10$ ). This suggests root translucency DAE methods are also applicable to the younger OpID cases.

In conclusion, the non-destructive DAE methods are informative when used to identify deceased individuals of Central American geographic origin.

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#### Dental Age Estimation, Root Translucency, Hispanic



## A32 Reevaluating the Use of Percentage of Bone for Histological Age Estimations

Mariah E. Moe, MA\*, New Braunfels, TX 78130; Timothy P. Gocha, PhD, Forensic Anthropology Center, San Marcos, TX 78666-4684

**Learning Overview:** After attending this presentation, attendees will understand the potential for using the percentage of non-remodeled bone tissue in the histological analysis of cortical bone for the estimation of age at death from human skeletal remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the use of non-remodeled bone allows for easier training of histological methods and provides a more time-efficient means for age estimation when compared to the more commonly used histological measures such as Osteon Population Density (OPD). In addition, this presentation will show how ArcGIS® software can be used to maintain accuracy and precision of data collection while allowing for ease of access for future analyses. Finally, this presentation will highlight the benefits of histology as an objective and highly sensitive tool that can be used in cases in which other macroscopic methods have failed or are unavailable.

Traditional macroscopic methods for estimating age at death from human skeletal remains can be highly accurate when applied to younger decedents but are notoriously inadequate when aging individuals over the age of 50 years. Primary, or non-remodeled, bone is produced during normal growth and development. Throughout life, individuals undergo the lifelong process of remodeling wherein primary bone is replaced with microstructures known as secondary osteons. As individuals age, the amount of primary bone tends to decrease as the number of osteons increases. Skeletal histology as a means for age estimation has the potential to overcome the challenges encountered with macroscopic methods and more accurately estimate age in older individuals.

This study quantified the percentage of primary bone from the anterior femoral midshaft of 30 modern cadaveric samples (15 males and 15 females) ranging in age from 21 to 97 years. All femora were digitally subdivided circumferentially into octants, and radially into periosteal, middle, and endosteal thirds. Specifically, the periosteal third of the anterior octant was examined, and the percentage of non-remodeled bone was evaluated using static images and direct microscopic observation in linearly polarized light as well as brightfield illumination. Quantification of the non-remodeled bone was accomplished using Geographic Information Systems (GIS) software, specifically the polygon feature tool within the ArcMap® interface of ArcGIS®. Polarized light assisted with the quantification of the primary bone in that it highlighted the parallel structure of this bone, clearly delineating it from the more concentric remodeled bone tissue. Often, a reversal line (the defining feature separating the secondary osteons and fragments from the surrounding primary bone) could be better visualized with fine adjustments of the slide using brightfield microscopy.

All statistics were performed using SPSS version 24 and/or 25. Independent samples *t*-tests demonstrated no significant differences between males and females based on age distribution. Normality of the data was evaluated using a Shapiro-Wilk test due to small sample size ( $n=15$ ) based on sex, where percentage of bone was the dependent variable and sex was the independent variable. The Shapiro-Wilk test revealed that the data were not normally distributed between the sexes nor with the sexes combined. The data were then transformed using the square root function in SPSS. Another Shapiro-Wilk test was performed and, as the test showed the transformed data to be normally distributed with maintenance of normality in the combined sex evaluations, all further analyses were conducted on the transformed percentage values with the sexes combined.

The data showed a statistically significant inverse relationship between percentage of non-remodeled (primary) bone and age at death. Regression analysis showed a linear relationship between the variables in the anterior, periosteal octant, accounting for 76% of the variability in age with a standard error of  $\pm 11.1$  years.

These results were compared to previous research conducted by Maat et al. that investigated a smaller Region Of Interest (ROI) and showed that increasing ROI size significantly improves its predictive power.<sup>1</sup> These results were also compared to the most widely utilized histological predictor of age, Osteon Population Density (OPD). Linear regression analysis of OPD data previously obtained by Gocha revealed a strong correlation between OPD and age, explaining 83% of the variability in age with a standard error of  $\pm 9.2$  years.<sup>2</sup> Quantification of the percentage of non-remodeled bone, however, requires less time and less training to implement than evaluating OPD and may be an adequate first step in histological analysis of age at death.

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### Histology, Age Estimation, Non-Remodeled Bone



### A33 An Overview of the Camp Fire and Wildfire Situation in the West

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**Learning Overview:** After attending this presentation, attendees will understand the scale and scope of the wildfire situation in the western United States and the critical need for planning for such future large-scale disasters.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of the preparation needed to access appropriate personnel, train teams in the identification of badly burned human remains, and establish support structures that facilitate the work on wildfire victims.

California, along with many western states in the United States, has experienced climatic changes with higher summer temperatures, a prolonged season without rain and, recently, winter rains that facilitated plant growth, thereby increasing the fuel load. These changes have been accompanied by increasing development of housing in once rural areas, often moving into heavily forested or wooded areas with limited access. These environmental and human factors have combined to produce conditions where wildfires can spread rapidly and have a fatal impact.

In recent years, California has seen a dramatic increase in wildfire deaths along with the acreage consumed. For example, in 2010 there were 17 wildfires over 1,000 acres in the state but no fatalities. In contrast, in 2017 there were 61 such fires and 34 deaths, and in 2018 there were 58 fires and 100 fatalities. Of the 20 largest wildfires on record in California, 15 have occurred since 2000. Likewise, 15 of the 20 most destructive fires (in terms of fatalities and structures destroyed) have also occurred since 2000. In the past, first responders were often those who died, but, increasingly, fires are sweeping through residential areas with little or no time to mount evacuations. The bulk of those dying have been trapped in houses or vehicles.

The Camp Fire, which started during a period of high winds in the early morning of November 8, 2018, epitomizes the problems. The fire swept through the small communities of Concow and Magalia but also demolished much of the city of Paradise. Limited egress trapped people in cars while the high number of elderly and/or disabled residents meant many were unable to flee. Those who could escape often did so with few possessions and limited means of communication, prompting frantic calls to the sheriff's office regarding missing relatives. In the aftermath, Butte County was faced with a huge number of "missing" and an unknown number of deceased in an area of more than 150,000 acres where downed power lines, ongoing fires, and poor communication systems were the norm.

As the frequency of fire deaths has increased, anthropologists, led by California State University (CSU) Chico, pathologists, odontologists, and other forensic scientists have developed strategies for search, recovery, and identification efforts that have proven to be effective and efficient. These approaches came into play with the Camp Fire and enabled systematic recovery and identification. Strategies balance the different contributions that members of the search and recovery teams bring along with the post-recovery analysis and identification. From this experience, lessons learned as well as potential pitfalls highlight the need for preparation, collaboration, coordination, delegation, and focus.

#### **Camp Fire, Wildfire Responses, Mass Fatality**



### A34 Butte County's Response to the 2018 California Camp Fire

Jennifer Celentano\*, Butte County Sheriff's Office, Oroville, CA 95965

**Learning Overview:** After attending this presentation, attendees will understand how the Butte County Sheriff's Office responded to the Camp Fire, now the deadliest wildfire in California history. This presentation provides information on the response operations of the Butte County Sheriff's Office.

**Impact on the Forensic Science Community:** The Camp Fire was the second of two back-to-back disasters in Butte County within 18 months. The first disaster took place in February of 2017 when the Oroville Dam Spillway failed. Knowledge gained from the spillway incident was subsequently applied when Butte County responded to the Camp Fire. This presentation discusses the lessons learned after responding to two mass disaster situations, but will primarily focus on the recovery efforts initiated in response to the Camp Fire, especially how Butte County navigated the ever-changing landscape of disaster response and the flexibility needed to adapt to unpredictable challenges.

The Northern California Camp Fire started around 6:15 a.m. on November 8, 2018, in a remote area of Butte County near the small community of Pulga in the Sierra Nevada foothills. From its origin, the fire was driven northwest by intense winds (up to 55mph). Jumping from ridge to ridge, it was estimated that the fire consumed approximately 80 acres per minute. The fire quickly pushed through the community of Concow. At 7:45 a.m., a spot fire was reported in eastern Paradise. By 8:45 a.m., the fire had progressed halfway through the town of Paradise and by noon, the California Department of Forestry and Fire Protection (CAL FIRE) had issued an order for fire resources to begin pushing cars off the roads to unblock escape routes. The fire continued to aggressively burn toward the surrounding communities of Oroville and Chico. At 9:08 p.m., fire personnel retreated out of Honey Run Canyon reporting that they "lost everything." In all, the communities of Concow, Paradise, Magalia, and Butte Creek Canyon were destroyed in less than 12 hours. Despite the danger and potential loss of life, Butte County first responders succeeded in evacuating 52,000 residents from the area.

Following the Camp Fire, a number of communication challenges arose. Dispatch centers became inundated with phone calls from people needing help or looking for loved ones. Coroner units were forced to use cell phones to communicate, but the fire had destroyed many cell phone towers, leading to widespread areas without cellular reception. Teams attempted to limit radio traffic to reduce the spread of information to the media.

As the magnitude of the incident became apparent, and the fatality count continued to rise, assistance was requested from throughout the state and neighboring states. Fifty-five of the 58 counties in California sent personnel to assist in recovery efforts. Search and Rescue (SAR) teams arrived from Oregon, Nevada, Texas, and Ohio. During the peak of the operation from November 9 to November 25, the daily personnel included 200 law enforcement officers, 500 SAR volunteers, 50 personnel assigned to SAR Overhead teams, 300 fire urban SAR personnel, 300 National Guard search personnel, 70 National Guard support personnel, 55 coroner investigators, 45 anthropologists, 4 forensic odontologists, 10 morgue personnel from Sacramento County, 5 DNA specialists, 3 crime analysts, 5 morgue support personnel, 3 pathologists, 25 canine units, one person from Alameda County doing aerial mapping, and approximately 200 miscellaneous support personnel.

As recoveries progressed, the focus shifted to decedent identification efforts. An ANDE® Rapid DNA machine was established at the Sacramento County Coroner's Office for victims' DNA. Butte County obtained next-of-kin DNA profiles to compare with the decedents' DNA profiles. By November 20, 17 of the 79 victims had been positively identified by DNA. One month later, by December 20, the fatality count had increased to 85 with 48 of the victims positively identified using DNA. Currently, nearly eight months later, the decedent count stands at 85 with 79 of the victims positively identified. Collaborative efforts are ongoing between Butte County and ANDE® to identify the remaining six victims.

Before it was ultimately contained on November 25, 2018, the Camp Fire consumed 153,336 acres and 18,793 structures, and resulted in the deaths of 85 people. To date, the Camp Fire is the deadliest and most destructive fire in California history, the sixth-deadliest wildfire in United States history, and the world's most costly natural disaster in 2018. From this fire, Butte County learned to navigate the ever-changing landscape of mass disaster response and how to best prepare for future mass fatality incidents.

#### Mass Disaster Preparation, Wildfire Victim Identification, Mass Fatality Response



### A35 Search and Recovery Protocols for Fatal Fire Victims of the 2018 California Camp Fire

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**Learning Overview:** After attending this presentation, attendees will understand protocols for the search and recovery of burned human remains from mass fatality fire scenes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of protocols used in the search and recovery of fatal fire victims from the 2018 Camp Fire disaster in northern California. This presentation will further discuss the need to adapt existing fire scene protocols to large-scale fire fatality events.

Fatal fire scenes are among the most complex and challenging contexts for forensic archaeological recovery. Thermally altered human remains are highly fragmentary and demonstrate a wide range of color changes, making it difficult to discern skeletal material from burned non-human remains and debris. Experimental research on fire scenes has resulted in detailed protocols for the efficient and thorough recovery of fatal fire victims. However, for large-scale mass fatality fire scenes, efficient processing of multiple residences requires a more flexible protocol, especially when the medicolegal focus is on victim recovery and identification.

In the early morning hours of November 8, 2018, high winds damaged power lines on Camp Creek Road in the community of Pulga, CA, resulting in a massive wind-fueled fire that devastated the adjacent rural communities of Paradise, Magalia, and Concow. The Camp Fire resulted in 85 fire-related fatalities and the destruction of more than 150,000 acres and 19,000 structures, making it the deadliest and most destructive fire in California's history. Although the search for and recovery of fatal fire victims was primarily coordinated through the Butte County Sheriff's Office, the effort enlisted the services of all available state coroner and search and rescue teams, the California Office of Emergency Services, the National Guard, the California Department of Forestry and Fire Protection (CAL FIRE), and multiple local and regional fire departments. Early in the recovery effort, the sheriff's office requested California State University, Chico's recovery team, which in turn requested additional anthropologists from California and Nevada.

The scale and complexity of the disaster required the deployment of several smaller teams to the three affected communities. The most effective team structure involved having anthropology team members embedded within coroner's teams, with CAL FIRE personnel available to assist with moving large debris from structures. Each day, the sheriff's office provided a list of residential addresses to search, based on reported missing cases. However, each team also received numerous call-outs throughout the day by search and rescue and canine teams to determine forensic significance during their systematic search of the 19,000 destroyed structures. The majority of the call-outs involved non-human remains (e.g., pets, wildlife, and food refuse), burned debris, or false-positive canine hits. Accuracy improved when anthropologists implemented training to assist searchers with recognizing burned and calcined remains. The size of the recovery team fluctuated significantly each day, requiring flexibility in team composition and selection of recovery scenes.

Over a 20-day period, forensic anthropology teams conducted targeted searches for human remains in hundreds of burned residential structures and automobiles. Search patterns began with a perimeter walk around the structure, followed by a minimally invasive foot survey through the structure with a small team, and finally, a thorough search with small hand tools using a larger team. This approach was efficient for locating remains, especially from trailer homes and one-story residences. Multi-story residences and apartment complexes required significant debris removal, as well as more in-depth excavation and searching to locate remains. In most cases, human remains consisted of small, calcined bone fragments, although residual charred tissue often adhered to the thorax, pelvis, and thigh region. Human remains were recovered using trowels, brushes, hand sieves, and other small hand tools, and screening stations were set up to ensure a thorough recovery of smaller remains. Coroner teams were responsible for scene documentation, photography, and recording the location of remains using a total station. In total, forensic anthropology teams assisted in the recovery of 75 of the 85 fire victims and, in some instances, assisted with secondary recoveries of scenes processed previously without anthropology teams. Three scenes involved multiple fire victims and showed extensive commingling. The recovery protocol implemented in the Camp Fire disaster was effective and efficient for locating and recovering all known fatal fire victims and further aided identification efforts by other forensic experts.

#### Forensic Anthropology, Mass Fatality Fire Scene, Burned Remains



### A36 Forensic Pathology, Identification, Morgue Operations, and Management of the Camp Fire Victims

*Kimbelry D. Gin\**, Sacramento County Coroner's Office, Sacramento, CA 95820; *Kathy Raven, MD\**, Sacramento, CA 95820

**Learning Overview:** After attending this presentation, attendees will understand some of the principles of forensic pathology utilized in examining wildfire victims, cause of death and certification, issues surrounding identifications, and the task of operating a morgue after a mass fatality event.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the issues faced and the solutions that were utilized when processing, examining, identifying, and certifying human remains after a wildfire. This presentation will also emphasize the need for a change in the way remains have been historically processed and examined to facilitate faster identification.

Mass fatality incidents are fraught with difficulties, from recovery to examination to identification. The larger the incident, the larger the issues encountered. On the morning of November 8, 2018, a wildfire, dubbed the Camp Fire, started in Butte County, CA, and quickly became the biggest mass fatality incident in California's recorded history for any wildfire. Butte County Sheriff Coroner requested assistance from Sacramento County with the examination and identification of the victims. The remains were transported to Sacramento County and were processed, examined, and various methods of identification were utilized. A team of Sacramento pathologists and two pathologists from surrounding counties examined 84 of the 86 victims. All examinations were completed within two weeks of the fire and the identification process began immediately. The causes of death ranged from fire-related injuries to gunshot wounds.

Within three months of the fire, 87% of the victims were positively identified despite the extremely poor condition of the remains. Five of the victims were able to be identified quickly by fingerprints; 15 were identified by dental comparisons; and 2 victims were identified by hardware. The majority of the fire victims were identified by rapid DNA, which had not been utilized in a mass fatality in the United States prior to this event.

The inclusion of rapid DNA into the identification process highlighted issues that had to be addressed. The conventional DNA testing, which is by nature a lengthy process, was replaced with rapid DNA, resulting in very quick identifications. Traditionally, DNA testing takes so long that the exams are completed and the causes of death are determined long before the DNA results are known so death certification is quick once the person is identified by DNA. With rapid DNA, the identification was completed before the cause of death was determined in most of the cases. This issue led to families having to wait to receive a death certificate to complete burial arrangements and complete federal paperwork for aid. Steps had to be taken to help expedite cause-of-death determinations and rushes were placed on toxicology in order to help the families with this process.

Another issue faced was the lack of anthropologists to handle examinations in the field and at the morgue. Some of the identification attempts were delayed due to this issue.

In the end, the lessons learned from this event will aid other agencies in similar mass fatality events. This incident is a real example of crossing borders with multiple agencies working together on so many levels, and the results were extraordinary.

#### Mass Fatality, Identification Methods, Forensic Pathology



### A37 Wildfire Search, Recovery, and Identification of Victims: Post-Recovery Anthropological Analysis and Identification

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**Learning Overview:** After attending this presentation, attendees will better understand the analytical and logistical challenges faced by anthropologists in the post-recovery analysis and identification of victims of a mass casualty wildfire. The primary goals of this presentation are to define the role of anthropology in this context and to present strategies for case documentation and maximizing evidence recovery.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing attention to the planning required for mass casualties from wildfire, which must include post-recovery analysis. Prior to any disaster, understanding the role anthropologists play in this context, how they integrate with the other team members, how to stage anthropological analysis within the identification process, and how and when to use anthropological services needs to be clarified and planned for.

The California wildfires tested the ability of agencies to plan and execute a systematic search, recovery, analysis, and identification of fire victims. As we plan for future fires, ensuring that there are personnel to cover the post-recovery operations is essential. Primary efforts are on positive identification of fire victims so that remains may be returned to grieving families as expeditiously as possible. The analytical and logistical challenges involved in post-recovery analysis and identification need to be addressed in order to smoothly integrate anthropology into morgue operations involving wildfire victims. This presentation offers procedures and strategies for the documentation and analysis of wildfire victims in the morgue setting.

Anthropologists are critical in the morgue analysis of wildfire victims, complementing the work of pathologists, odontologists, DNA analysts, and morgue staff. Anthropology's contributions include: (1) removal of non-osseous material from human remains, (2) separation of human and non-human material, (3) identification of incomplete recoveries, (4) identification of split recovery of remains, (5) identification of commingled remains, and (6) identification of elements useful for positive identification, such as appropriate DNA samples. Using examples from the Camp Fire, handled through the Sacramento County Coroner's Office, basic issues include the range of thermal damage to remains, the extent of commingling (human and non-human), and the difficulty of isolating material useful for identification.

In the Camp Fire analysis, anthropologists examined 69 of the 86 "cases" recovered. While some remains were partially burned, retaining significant soft tissue, most cases were severely impacted by the fire. Sorting calcined material became extremely important and resulted in fluctuating body counts as body segments were reunited or commingled remains identified.

Anthropologists began work in the morgue approximately nine (9) days after the outbreak of the fire. Often their analyses followed examination by the pathologists and odontologists. This sequence was problematic as anthropologists focused not only on the numbered remains, but also on the elements represented by addresses and the localities of where remains were recovered. Often commingled remains recovered from the same address were bagged "separately" but each container collected from the field represented a full intermingling of bodies from a specific locale.

In order to work efficiently, a systematic approach was developed. Bone fragments were separated by body segment, examined to ensure that full body recovery was present, and elements separated that may be useful for positive identification. Additionally, all remains found at a single address were analyzed simultaneously to address comingling issues. Peer review was accommodated by separate examination by one anthropologist, then cross-checking the work of the other. Potential disagreements or clarifications were discussed prior to sign-off. Case records were memorialized using extensive photo documentation and standard recording forms.

Given that major contributions of anthropology include identifying teeth, comingling, and appropriate samples for DNA, the integration of anthropology at the beginning of the analytical process is recommended. With these fragile remains, anthropologists should work prior to other disciplines (pathology, odontology, and DNA) to minimize damage and to maximize appropriate specimens for other experts. Another important lesson learned is the importance of small bones, such as carpals and tarsals, and bone fragments to identify comingling. Lastly, to prepare anthropologists for the complexity of burned bone analysis on a large scale, training opportunities are recommended to address these issues as a specialty area.

It is recommended that planning for future such disasters account for early anthropological analysis in the post-recovery efforts. Anthropologists should work on fully burned remains prior to the work of the pathologists and odontologists. Normal analysis is limited by the condition of remains and pace of identification, but valuable contributions are still possible. Training specific to burned bone analysis is recommended for anthropologists planning on contributing to wildfire mass disasters.

#### Forensic Anthropology, Burned Bone, Identification





## A38 Wildfire Search Protocols and Victim Recovery

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**Learning Overview:** After attending this presentation, attendees will better understand the use of Rapid DNA for disaster victim identification and the integration of Rapid DNA identification with forensic odontological, pathological, and anthropological analyses. The primary goal of this presentation is to discuss the value of routine, coordinated utilization of Rapid DNA identification during and in the immediate aftermath of a mass disaster.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the use of Rapid DNA to generate DNA IDs (also known as DNA fingerprints or Short Tandem Repeat (STR) profiles) in real-time from unidentified human remains. By comparing a database of these DNA IDs to those generated from familial reference samples and antemortem victim samples, it is possible to assign identities to victims, often within hours of intake.

In November 2018, the Camp Fire raged through Butte County, consuming 800 acres of Northern California per minute. As with most mass disasters, conventional identification modalities (e.g., fingerprints, dental records, radiographic features, hardware) were utilized to identify victims. The intensity and duration of the fire severely degraded most of the remains, and, as a result, the conventional approaches were useful in only about one-quarter of the cases. In the past, the remaining cases would be subjected to laboratory DNA analyses, which typically requires months to years. Instead, Rapid DNA identification, was, for the first time, utilized to identify the majority of victims within hours to days following recovery.

Rapid DNA identification is the fully automated process of generating a DNA ID from a forensic sample, typically performed outside the laboratory by non-technical operators, with results available in less than two hours.<sup>1</sup> Rapid DNA identification is based on four integrated processes: purification of genomic DNA from a sample; rapid multiplexed amplification of the FlexPlex™ loci, a set of 27 STR loci (amelogenin, 3 Y-chromosomal STR (Y-STR) loci, and 23 autosomal loci); separation of the resulting STR amplicons by polyacrylamide gel electrophoresis; and data processing and locus and allele assignment using an onboard expert system.<sup>2</sup> DNA IDs generated from human remains were compared to DNA IDs generated from family reference samples using automated kinship software.

Rapid DNA identification expanded to take on a central role in victim identification. Faced with burn victims, among the most difficult types of remains to identify, the use of Rapid DNA led to surprising synergies, with work being performed in parallel in pathology, odontology, and anthropology. From the use of DNA IDs to triage efforts in these disciplines to the identification of comingled remains, Rapid DNA was shown to be an invaluable tool in the victim identification process. This presentation will describe results from the rapid processing of severely degraded bone, tissue, and blood samples and the use of familial searching software. Finally, the application of Rapid DNA in related forensic applications will be discussed.

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### Rapid DNA Identification, FlexPlex™, Familial Searching



### A39 Outside Agency Response to the California Camp Fire

*Timothy P. Gocha, PhD\**, Forensic Anthropology Center, San Marcos, TX 78666-4684; *Kyra E. Stull, PhD\**, University of Nevada, Reno, Reno, NV 89557; *Marin A. Pilloud, PhD*, University of Nevada, Reno, Reno, NV 89557-0096

**Learning Overview:** After attending this presentation, attendees will understand the ways in which anthropologists from agencies outside the state of California responded to offer assistance in the search and recovery efforts in the aftermath of the Camp Fire in northern California.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering insight into the logistical, financial, and ethical considerations faced by outside agency responders and offer recommendations for other anthropologists who may one day serve as responders from outside agencies to mass fatality incidents.

On the morning of November 8, 2018, a wildfire started in Butte County in northern California and began to spread. Termed the Camp Fire, it quickly grew to become the most destructive and deadliest wildfire in California history. By the evening of November 10, anthropologists responding to the incident from California State University, Chico recognized the need for additional anthropological resources and requested assistance from forensic anthropologists in northern and southern Nevada. Data for this presentation comes from the experiences of these forensic anthropologists. Similarities and differences will be noted and discussed.

Anthropologists responding from northern Nevada were primarily affiliated with the Department of Anthropology with the University of Nevada, Reno (UNR), including 2 faculty members and 15 graduate students. Due to their proximity to the camp fire, UNR anthropologists were able to begin assisting with recovery efforts on Sunday, November 11. They were also able to return to Reno to fulfill academic obligations during the week and return to assist as available. In total, UNR anthropologists deployed three times throughout the month of November to provide support over approximately nine days. The large number of individuals displaced by the wildfire resulted in no vacancies in local hotels in Chico and all UNR anthropologists stayed in personal residences of Chico State anthropologists. Financially, efforts from UNR anthropologists were initially self-financed, though students and faculty were eventually reimbursed for gasoline and some food expenses through UNR using funds received from local forensic anthropological casework.

Anthropologists responding from southern Nevada were affiliated with the University of Nevada, Las Vegas (UNLV), including one faculty member and three graduate students. However, in their response to the Camp Fire, these anthropologists were considered primarily affiliated with the Clark County Office of the Coroner/Medical Examiner (CCOCME), where they served as the forensic supervisor, two of the graduate students were part-time forensic staff, and one graduate student was a forensic anthropology intern. Because of their affiliation with a government agency, anthropologists from the CCOCME were subject to an Emergency Management Assistance Compact (EMAC) agreement between California and Nevada, which delayed deployment to the Camp Fire response. As such, CCOCME anthropologists were unable to respond as quickly as those from UNR and began assisting with recovery efforts on Monday, November 12, and stayed until November 17. Similarly to the anthropologists from UNR, CCOCME anthropologists also relied on Chico State anthropologists for lodging, and efforts were also initially self-funded. Although the EMAC initially delayed CCOCME response, it did allow for those anthropologists to be paid for their time and efforts in the field, as well as reimbursement for travel expenses by Clark County, which was later reimbursed by the state of California. Further conversations after the event with EMAC coordinators revealed that while EMAC does not have provisions for academics per se, if anthropologists are employed by a public university, accommodations could be made to ensure reimbursement for those individuals. It is recommended that anthropologists housed in academic units begin a dialogue with Emergency Management coordinators in their state prior to a mass fatality event, to establish a framework for reimbursement.

The response by anthropologists from both UNR and the CCOCME offered not only an important professional service to the affected communities, but also allowed for hands-on training and professionalization of students in the field. This ranged from practical experience of field operations in a series of changing environments to building skills of professional interaction with various law enforcement and responder agencies.

#### Camp Fire Response, Search and Recovery, Mass Fatality Response



## A40 Demography of the Camp Fire Fatalities

*Samuel A. Mijal, BS\*, Phoenix, AZ 85004; P. Willey, PhD, Chico State University, Chico, CA 95929-0400*

**Learning Overview:** After attending this presentation, attendees will understand which segments of a population are disproportionately impacted during catastrophic events. Such was the case involving foothills communities devastated by the November 2018 Camp Fire in northern California. There, older adults and those with mobility-inhibiting disabilities died in greater proportions than other groups.

**Impact on the Forensic Science Community:** This presentation will impact the forensic sciences community by serving as a case study for identifying high-risk groups in wildfire mass fatality events. Employing these results and insights as a foundation, the most at-risk groups can be identified and slated for education, planning, and warning before catastrophic events occur. In addition to preparations, those at-risk groups should be the focus of resources during emergencies, as well as search and recovery and identifications following catastrophes.

Data for the Camp Fire Communities (Concow, Paradise, and Magalia) came from several sources. The 2010 United States census provided the communities' age and sex distributions. Economic and disability information were derived from the 2017 American Community Survey estimates. The Butte County Sheriff's Office provided the fatalities' ages (recorded to the most recently completed whole year), sex, and locations. Information concerning the victims' disabilities came from a local newspaper's accounts consisting primarily of personal recollections from family members and friends.

Approximately 38,000 inhabitants lived in the three Camp Fire Communities, with the largest town (Paradise) comprising about two-thirds of that total. Stereotyped as retirement communities, the foothill towns tended to have greater proportions of older adults, and many inhabitants lived on limited incomes in a community where the median income was \$10,000 below the national median and \$19,000 below the state medium (Community median income was \$47,743). In addition, these towns had a greater proportion of disabled community members. As examples, the Communities accounted for nearly double the rate of ambulatory and independent living-challenged members than the state or country.

Eighty-five individuals died in the Camp Fire, 83 of whom have been identified. This demographic work uses the identified individuals and omits the other two from further consideration. No infants, children, or adolescents died from the blaze. In addition, very few young and middle-aged adults expired. On the other hand, older adults represented a disproportionately high number of victims. The ages of the fatalities varied from 20 to 99 years, with a mean of 72.06 years. By sex, female fatalities exceeded male fatalities: 44 females, 39 males. The fatality age distribution by sex proved statistically similar. However, when four outliers were excluded from the data set (two younger outliers of each sex), a statistically significant difference occurs. Female fatalities averaged five years older than males. The ancestral makeup of the Camp Fire Communities was largely White (92%), with 6.94% self-identifying as Hispanic. Ten (12%) fatalities had Spanish surnames, a distribution greater than the census percentage and approached, but failed to achieve, statistical significance. Anecdotal evidence provided by personal accounts indicated that Camp Fire victims exhibited high rates of mobility-inhibiting disability.

The towns of Concow, Magalia, and Paradise represented communities with higher-than-average at-risk populations with advanced age and mobility-inhibiting disability being the main issues. Assessment of the Camp Fire victims' and Communities' demographic variables reinforced how these risk factors contributed to individual casualties.

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### Demography, Camp Fire, Mass Fatality Event



## A41 Locational Analysis of the Camp Fire Fatalities in Communities and Residences

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**Learning Overview:** After attending this presentation, attendees will understand the application of Geographic Information Systems (GIS) to forensic anthropology for the spatial analysis of fatalities in a mass disaster.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the use of geospatial analysis to examine mass fatality events to assist communities in identifying vulnerability in a population and to aid in recovery efforts.

On Thursday, November 8, 2018, a brush fire in Pulga, CA, started early in the morning. Fueled by high winds and overall dry climate from significant droughts, the fire swept through the towns of Concow, Paradise, and Magalia. These towns are all foothill communities, with Magalia and Paradise having one arterial road, named the Skyway, to get in and out of the town. The Camp Fire is currently the deadliest and most destructive fire in California's history. The fire burned more than 150,000 acres, more than 18,000 structures were destroyed, and it resulted in 85 fatalities. With the increase of fire events, it is important to examine disasters to identify vulnerable individuals in communities in order to help prevent fatalities in future mass disaster events.

This analysis utilizes ArcMap® 10.7 to spatially examine the locations of the fatalities from the Camp Fire. Data on fire victims and the locations where these individuals were recovered was provided by Butte County Sheriff's Office. Information concerning the victims' disabilities came from local newspaper accounts that consisted primarily of personal recollections from family members and friends. The fire perimeter used for this analysis was obtained from the California Department of Forestry and Fire Protection (CAL FIRE) website. Shapefiles for roads and counties were downloaded from United States census data. Landsat 8 satellite imagery was also utilized.

GIS analysis allows for assessment of factors that could have contributed to the deaths of individuals in the Camp Fire. This includes the location of fatalities in relation to the spread of the fire across the communities. The victims' ages, sex, and any mobility-inhibiting disabilities are also assessed. Furthermore, the distance from home addresses to where victims were found in vehicles on arterial and secondary thoroughfares is evaluated to assess the relationship between the human response to the fire spreading and to evaluate how traffic congestion in the early hours of the fire contributed to the high fatality rate for this fire.

Results from spatial analysis led to several conclusions. Primarily, it is concluded from this study that proximity to arterial thoroughfares did not influence the Camp Fire victim demographics. In addition, this study identified a lack of relationship between age and distance of remains from the origin of the fire, contrary to expectations. This suggests victim age and disability played a significant role in victim demographics, while distance from the fire's origin and the warning time it afforded was less of a factor. Finally, sex of the individual and distance from the origin of the fire were found to have no relationship, supporting the conclusion that sex was not a strong factor in the response to the fire and subsequent evacuation of the Camp Fire communities. A spatial autocorrelation, using Moran's I to examine fatalities and the variable of age, shows a significant positive relationship for a clustered pattern in the distribution of fatalities. This supports victim age as playing a role in victim demographics and indicates that individuals are likely not randomly dispersed throughout the towns of Concow, Paradise, and Magalia.

Geospatial examination of fire fatalities, as applied in this study, can allow for identifying preventative measures for communities that are particularly at risk and vulnerable in mass disaster events. Also, the examination of location patterns in fatalities could aid in future mass disaster recovery efforts of human remains.

### Mass Fatality Incident, Spatial Analysis, Camp Fire



### A42 Anthropology and Mass Fatality Management in Wildfire Disasters

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**Learning Overview:** After attending this presentation, attendees will better understand the roles anthropologists play in managing both recovery and identification of victims from wildfire events. Wildfires are a type of mass disaster that requires the coordination of resources across numerous agencies to address fatality management.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of mass fatality policy development in response to lessons learned from mass disasters. The continued development of mass fatality policies affects both training and agency responses to future events.

The 2018 wildfire season in California was the deadliest and most destructive season in California Department of Forestry and Fire Protection (CAL FIRE) history. With a total of 310 incidents, California recorded 93 fatalities and more than 23,000 structures destroyed. The deadliest single incident of both the season and California wildfire history was the Camp Fire in Butte County, CA. The Camp Fire alone recorded 85 fatalities to date and more than 18,793 structures destroyed. Moving through the communities of Pulga, Concow, Paradise, and Magalia, the wildfire's speed and size also required the largest deployment of resources to a single mass fatality event in California history.

Mass fatality incidents are defined as any incident in which the number of dead immediately overwhelms local resources. In California, the local agency maintains operational control but can request assistance from both state and federal agencies in the event of a disaster, as well as other jurisdictions through a mutual aid system. Assistance can include subject matter experts, funding, and resources. Forensic anthropologists are often called to assist in both the recovery and identification process of victims in mass fatality incidents, along with medical examiners, coroners, odontologists, X-ray technicians, and other mortuary personnel. Traditionally, forensic anthropologists play a larger role during mass fatality disasters in the mortuary identification process than in field recovery operations. However, the 2018 Camp Fire utilized more anthropologists in field operations than in mortuary operations, inverting the more typical use of anthropologists. This resulted in the recovery of 85 victims across a 153,336-acre search area in under three weeks with no false positive cases sent to the mortuary.

Terrorism events, aviation disasters, pandemics, earthquakes, and hurricanes are usually the focus of mass fatality planning, whereas wildfires have not received the same amount of attention. However, the fatalities associated with wildfires present unique challenges to both the recovery and identification processes. Lessons from the 2018 fire season highlight the need to include anthropologists in operational planning in both pre-planning and incident response. This includes organization of victim search efforts, distinguishing between human/non-human/non-osseous materials, assistance in mortuary operations, field gear acquisition, field training for all search personnel, data logistics, and multi-agency coordination.

This study recommends an increased role for anthropologists in the pre-planning stage to increase the training sources available to law enforcement, search and rescue, CAL FIRE personnel, and medical examiner/coroner teams. In addition, involvement in planning would help with preparatory field gear acquisition for more rapid deployment during wildfire events. Also recommended is the inclusion of anthropology personnel in the active search for victims of wildfires, given the condition of remains in these instances. This recommendation reduces the number of false positive numbers in the field and increases the percentage of remains recovered, which in turn improves the speed and accuracy of positive identifications in the event.

#### Camp Fire, Disaster Planning, Wildfire Responses

## A43 The Infant Injury Database

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**Learning Overview:** After attending this presentation, attendees will be aware of the Infant Injury Database (IID), the types of data archived in the database, the composition of the population sample, and the mechanism for requesting access to the data. This presentation provides a more detailed description of the database previously described by Soto Martinez et al.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by creating awareness of and providing information about a valuable resource for conducting evidence-based research on the recognition and diagnosis of child abuse.

The IID is a valuable data source for forensic and clinical practitioners interested in developing statistically sound, validated diagnostic models to inform decision-making with regard to abusive injury. It is a comprehensive collection of demographic information, investigative information (law enforcement, medical examiner death investigation, child protective services), scene observations, medical history, and autopsy findings from all pediatric (0–5 years) examinations conducted at the Harris County Institute of Forensic Sciences (HCIFS). To mitigate the introduction of selection bias, age was the only factor limiting inclusion into the IID. All data pertaining to Postmortem Examinations (PME) were prospectively collected from autopsy reports and gross rib exams or full pediatric skeletal exams conducted by an HCIFS forensic anthropologist. Full pediatric skeletal exams were conducted, per the pathologist's request, on cases of concern for abusive injury or suspicious circumstances.

For each case in the database, 2,071 fields of data (discrete and free-text) are collected with the ability to attach additional comments to any data field. General case information, such as demographics, height/weight, and manner/cause of death, is documented in the first 25 fields of the database. Ninety-nine fields describe the events leading up to the terminal event (i.e., co-sleeping, napping, traumatic injuries), and detail information such as who found the decedent (i.e., mother, father, parent's paramour, etc.) and where they were found (i.e., bed, bassinet, crib, couch). These fields also describe the decedent's physical appearance (i.e., lividity, rigor mortis, cold to the touch) upon discovery or assessment by medical personnel. If the decedent was not transported to the hospital, observations from scene photos are recorded in 27 fields, describing the decedent's sleep environment (when applicable) and general home environment (i.e., clean, unkempt, insect activity, etc.). Eighty-seven fields document the decedent's medical history (prenatal, birth, and postnatal) and family medical history. Parental/caregiver social histories (tobacco, alcohol, and illicit drug use) are documented in 21 fields. History with child protective services involving the decedent, sibling, and/or parent/caregiver are recorded in 157 fields, including number of investigations, associated allegations, and allegation determination. Medical examiner findings are recorded in 1,655 fields and are organized by location of injury, such as external body, internal body, and skeletal system. External injuries to specific body parts (i.e., face, lips, left hand, buttocks, etc.) are documented in 76 fields, including injury type (i.e., contusion, laceration, abrasion, etc.) and whether the observed injuries resulted from medical intervention. There are 106 fields for documenting internal injuries to body organs, and subcutaneous and intramuscular soft tissues, which includes descriptions of injury type (i.e., hemorrhage, pulpification, laceration). The largest number of PME fields ( $n=1,473$ ) are dedicated to the documentation of skeletal injuries. There are fields for recording fracture type, number of fractures, and stage of healing for almost every bone in the body. Free-text boxes are utilized for documenting injuries to the bones of the hands and feet as these types of injuries are uncommon.

Currently, the IID contains data on 710 pediatric cases (female=289, male=421) and the sample continues to expand. These data were collected intermittently from 2010 to 2013 and have been continuously collected from 2014 to the present. Infants form the largest group in the IID (<12 months=633, 89%), followed by children 1–2 years of age ( $n=32$ ), 2–3 years of age ( $n=22$ ), 3–4 years of age ( $n=15$ ), and 4–5 years of age ( $n=8$ ). With regard to ethnicity, African Americans form the largest group in the IID ( $n=305$ , 43%), followed by Hispanics ( $n=237$ ), White/European Americans ( $n=150$ ), and Asians ( $n=18$ ).

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### Pediatric, Injury, Databases



## A44 Pediatric Posterior Rib Terminus Defects: An Investigation of a Potential Mimic of Traumatic Injury

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**Learning Overview:** After attending this presentation, attendees will be aware of rarely identified mimics of posterior rib head fractures that are inconsistent with inflicted trauma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that posterior rib head fracture mimics are a fairly common finding and should be interpreted cautiously.

Posterior rib fractures are considered highly suspicious for abusive injury in infants due to the mechanism by which these injuries occur.<sup>1</sup> Numerous studies demonstrate an association between posterior rib fractures and inflicted injury in infants. While this association is clear, recent casework at the Harris County Institute of Forensic Sciences (HCIFS) requires that at least some posterior rib defects be considered in the context of growth and development as well as the presence and/or absence of other injuries and/or risk factors. At the HCIFS, standard autopsy protocol includes an *in situ* rib exam on all pediatric cases less than five years of age. In 55% ( $n=41$ ) of the 75 cases examined since January of 2019, at least one subtle linear defect was observed on the pleural surface of one or more ribs near their attachment to the vertebral column. Grossly, these defects have morphological characteristics suggestive of advanced healing, such as bone formation along the endosteal portion of the fracture surface. Similar defects have been previously described by Kemp as clefts that are “triangular-shaped defects in the primary spongiosa with the tip at the growth plate and the base at the periosteum.”<sup>2</sup> Kemp notes that the clefts are filled with an “amorphous, granular, eosinophilic acellular material of uncertain origin,” which was previously described by Dolinak and Matshes.<sup>2,3</sup> The exact content of the material is unknown, but Kemp hypothesizes the material is a response to inflammation and repair and contributes the clefts to microscopic fractures.<sup>2</sup> However, the characteristics of some of the pediatric cases at HCIFS complicate the interpretation of these defects. The defects were observed in one fetus and in several infants less than one week of age, as well as in infants with no other evidence of injury. Additionally, histological examination of these defects shows no cellular evidence of healing. As a result of these complications, a closer examination of the context in which these posterior defects are observed is warranted.

Manner and cause of death had been classified for 19 of the 41 cases with posterior defects. Cause of death was unrelated to trauma for 16 of the 19 cases. Only one of the three cases with a trauma-related cause of death was suspicious for abusive injury (Manner—Undetermined, Cause—Blunt force head trauma). Of the 41 cases with posterior defects, 16 had no other skeletal injuries, including rib fractures, and 17 had rib fractures but no other skeletal injuries. The fracture pattern in most of the cases with rib fractures was consistent with cardiopulmonary resuscitation.

A logistic regression model was used to examine the relationship between the presence of posterior defects as the dependent variable and age, sex, ethnicity, the presence of other rib fractures, and the presence of other skeletal injuries (excluding rib fractures) as the independent variables. The presence of posterior rib defects was significantly related to sex ( $p<.001$ ). The odds that females had posterior defects was 7.04 greater than males ( $OR=7.04$ , 95% CI [2.27, 21.83]). Age may also have a significant effect on the presence of posterior rib defects ( $p=.058$ ). The odds of observing posterior defects in older children were lower than in younger infants ( $OR=0.96$ , 95% CI [0.92, 1.00]). Age is statistically significant when entered as the only independent variable in the model ( $p=.047$ ). Neither ethnicity, the presence of other rib fractures, nor the presence of skeletal injuries had a significant relationship with the presence of posterior defects ( $p>.05$ ). These relationships are inconsistent with the general trends observed among the infants autopsied at HCIFS that die of abusive trauma or traumatic injury. The odds of traumatic death ( $OR=1.08$ , 95% CI [1.06, 1.10]) and abusive injury ( $OR=1.06$ , 95% CI [1.03, 1.08]) increases as age increases. Sex had no significant relationship with causes of death related to trauma or abusive injury ( $p>.05$ ).

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2. Kemp, Walter Loren. *Microscopic Examination of Rib Heads: A Useful Adjunct in the Investigation of Infant Deaths*. PhD diss., University of Montana, 2014.
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### Child Abuse, Posterior Rib Fractures, Trauma Mimics



## A45 Evaluating Bone Fracture Healing and Variability Across Forensic Samples Using an Anabolic/Catabolic Model of Bone Repair

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**Learning Overview:** The goal of this presentation is to evaluate bone fracture healing and the staging systems that are currently used to estimate its progression and timing in a forensic context. Microscopic and macroscopic comparisons of healing fractures across a diverse forensic sample are used to document variability in timing and character of bone healing and illustrate the inadequacies and errors associated with current models and stages of bone repair. This variability is interpreted through a newer anabolic and catabolic model of bone repair focused on the coordinated and integrated processes of osteoblastic-driven bone deposition and osteoclastic bone resorption.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that research will enhance forensic scientists' recognition of variability in the bone fracture healing process and its outcomes as well as the factors that produce and influence this variability. Researchers and clinicians will recognize the importance of comorbidities for fracture repair and will be cautioned that reliance on the application of previously published fracture healing estimates across forensic samples of varying age, fracture and trauma type, and health may lead to inaccurate Time Since Injury (TSI) estimates and interpretations. Use of a more fluid and integrated anabolic/catabolic model for bone repair will aid in more accurate interpretation of bone fracture healing (including TSI).

Interpretation of non-accidental pediatric and elderly injury is often dependent upon evaluation of previous bone injury and its repair process and timing. Currently, a myriad of bone healing schemata have been published, with little understanding of their application to forensic contexts. It is hypothesized that demographic (e.g., age), biomechanical (e.g., fracture and trauma type and location), and biomedical (e.g., treatment of fracture, presence of comorbidities) factors significantly influence bone healing and complicate and, in some cases, nullify the use of these published staging systems in a forensic context.

This hypothesis will be tested by first conducting a comparative review of published bone fracture healing staging systems across major forensic and biomedical sources dating back to 1980. Bone healing schemata variables compared include stages of healing presented, terminology used to describe the healing process, timetable for repair, and modality on which the stages are based. These staging systems are then applied to the forensic fracture sample from the Radford University Forensic Science Institute's Antemortem Fracture Archive (RUFSA AFA). This forensic sample consists of more than 250 antemortem fractures of varying type and location derived from (currently) 20 individuals ranging in age from 27 days to 92 years. It contains more than 3,000 macroscopic and microscopic (5x–200x magnification, using a 3D digital light microscope) images illustrating all phases of the bone repair process.

Results of this study identify at least 13 distinct bone healing staging systems that have been applied to fracture healing interpretations in a forensic context. All, to some degree, derive from radiographic assessments of bone repair across a variety of cohort demographics, fracture and trauma types, and locations from non-forensic contexts. They employ inconsistent fracture repair terminology, rely on rigid classification systems, and consider the bone healing process a categorical progression through finite and mutually exclusive stages. Application of these published staging systems to the diverse forensic fracture sample produces widely varying and unclear characterizations of bone healing and its timing and interpretation.

Utilization of a microscopic anatomy-based anabolic/catabolic model based on a continuous, coordinated, and integrated process of bone repair involving osteoblastically driven bone deposition and osteoclastic bone resorption produces more accurate results. This model has the advantage of flexible adaptation to documented variability in demographic, biomedical, and biomechanical influences on bone fracture.<sup>1,2</sup>

It is concluded that both macroscopic and microscopic evaluations of bone healing are important components of fracture interpretation, as is the recognition of the significant effect of many variables upon the fracture repair process and timing. This research illustrates well the 2020 AAFS theme of *Crossing Borders*—the use of an interdisciplinary (in this case, biomedical and anthropological) approach and model produces more accurate interpretations of bone fracture healing, its outcome, and interpretation in a forensic context.

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### Antemortem Fracture, Bone Healing, Comorbidities





## A46 Anthropological Examinations of the Hyoid, Thyroid Cartilage, and Cricoid Cartilage in Cases of Possible Neck Trauma

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**Learning Overview:** After attending this presentation, attendees will understand the importance of evaluating the thyroid and cricoid cartilages, in addition to the hyoid, in cases of possible neck trauma. Attendees will also understand fracture patterns with respect to hyoid fusion, manner of death, and presence of soft tissue hemorrhage.

**Impact on the Forensic Science Community:** This retrospective study will impact the forensic community by providing data for fracture patterns of the hyoid, thyroid cartilage, and cricoid cartilage analyzed by anthropologists in a medical examiner's office.

The hyoid, thyroid cartilage, and cricoid cartilage are critical in trauma interpretation as fractures of the neck organ block are commonly associated with strangulation, hanging, and direct blunt force. Between 2001 and 2019, the Forensic Anthropology Unit (FAU) at the New York City Office of Chief Medical Examiner evaluated 187 cases in which some combination of the hyoid ( $n=168$ ), thyroid cartilage ( $n=89$ ), and/or cricoid cartilage ( $n=77$ ) were submitted for an anthropological analysis of possible peri-mortem blunt force trauma. The FAU protocol for examining these cases begins by radiographing the removed specimen in standard Antero Posterior (AP) and AP oblique views to visualize the horns. The hyoid is then macerated. The larynx is fixed in a 10% buffered formalin solution and the soft tissues and perichondrium are carefully removed with small forceps and a scalpel, often under magnification. Data from these cases has been retrospectively compiled to assess interactions between fracture location and frequency, age, fusion of the hyoid, manner of death, and presence of hemorrhage in the surrounding soft tissues.

Of the 168 hyoids evaluated, there were 66 fractures noted on 54 cases. Fractures of the hyoid were categorized by location: right greater horn midpoint ( $n=28$ ), left greater horn midpoint ( $n=25$ ), right joint ( $n=6$ ), left joint ( $n=7$ ), right posterior end ( $n=0$ ), left posterior end ( $n=0$ ), and the body ( $n=0$ ). Greater horn midpoints were the most frequently fractured location (80.3%) with no side preference. Similarly, no relationship could be determined between the fracture location and the state of fusion. The fracture side frequency was similar in cases where the greater horns were bilaterally fused and bilaterally unfused. When cases were unilaterally fused, there were six fractures on the fused side and four fractures on the unfused side. The average age of individuals with bilateral unfused greater horns was younger than those with the left, right, and both greater horns fused (39.7 versus 50.5, 54.8, and 55.2, respectively) but a significant relationship between age and state of fusion was not identified. The youngest individual exhibiting bilateral fusion was 27, while the oldest individual exhibiting bilateral non-fusion was 88.

Of the 89 thyroid cartilages evaluated, there were 82 fractures noted on 49 cases. Fractures of the thyroid cartilage were categorized by location: right superior horn ( $n=16$ ), left superior horn ( $n=19$ ), right base of the superior horn ( $n=11$ ), left base of the superior horn ( $n=12$ ), right inferior horn ( $n=3$ ), left inferior horn ( $n=4$ ), right lamina ( $n=5$ ), left lamina ( $n=8$ ), and midline lamina ( $n=4$ ). Of the 77 cricoid cartilages evaluated, there were 29 fractures noted on 20 cases. Fractures of the cricoid cartilage were categorized by location: midline ( $n=3$ ), right of midline ( $n=8$ ), left of midline ( $n=13$ ), right lateral ( $n=3$ ), and left lateral ( $n=2$ ). No fracture side preference was noted on either the thyroid or cricoid cartilages.

In total, at least one fracture was observed on nearly half of the cases (91/187). Trauma was noted on about half of the cases certified as homicide or undetermined. Suicides were more likely to exhibit trauma whereas accidents and natural deaths were less likely. The presence of hemorrhage in the surrounding soft tissues was slightly more likely to be associated with an underlying fracture (66 with trauma, 45 atraumatic). A lack of hemorrhage was about twice as likely to be associated with no trauma (36 atraumatic, 17 with trauma).

Of the 187 cases, 70 had both hyoid and laryngeal cartilages present for evaluation. Twenty-seven exhibited no trauma while 14 exhibited fractures of both bone and cartilage. Only 1 hyoid exhibited trauma when the cartilages were atraumatic, yet 28 cartilages exhibited trauma when the hyoid was atraumatic. This contrast underscores the importance of examining the thyroid and cricoid cartilage, in addition to the hyoid, in cases of possible neck trauma.

### Hyoid, Larynx, Blunt Force Trauma



### A47 Utilizing Reflectance Transformation Imaging (RTI) for the Analysis of Saw Mark Characteristics on Kerf Walls: A Comparison of Traditional Imaging Techniques.

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**Learning Overview:** After attending this presentation, attendees will have some understanding of what RTI is and how to use it for analysis, the traditional approaches to saw mark analysis, and the potential advantages involved in including RTI in the forensic analysis of saw marks on bone.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting that RTI, an inexpensive and easy-to-use technology, can be used for saw mark analysis with comparable results to traditional methods and, in some cases, can more greatly enhance characteristics. RTI has the potential to expand upon traditional analytical techniques and ameliorate imaging difficulties associated with more traditional methods.

RTI is a computational photography technique which documents and enhances the 3D reflectance properties of the surface of an object. A series of digital photographs are taken from a stationary camera position while the light source is projected from a different known angle. These photographic sequences are synthesized as Polynomial Texture Maps (PTM) and can be viewed much like a conventional 2D photographic image. However, unlike a traditional photograph, a PTM is derived from the 3D properties of the object, allowing the user to virtually re-light the object using the designated viewing software. Although RTI has primarily been used in the cultural heritage sector, it has recently transitioned into the realm of forensic science.

Traditionally, saw mark analysis is completed macroscopically, with low-powered stereomicroscopes or, more recently, Scanning Electron Microscopes (SEM). To assess the viability of using RTI for saw mark analysis, three different handsaws were used to fully section porcine radii, resulting in 42 kerf walls. All kerf walls were examined with the three traditional methods, as well as with RTI, utilizing a saw mark analysis methodology derived from Symes et al.<sup>1</sup>

The results demonstrate that RTI has a greater capacity for digital alteration and examination than the traditional methods, with statistical analysis demonstrating comparable accuracy. Macroscopic examination resulted in a lack of identification of kerf wall characteristics due to the lack of magnification used for analysis. Characteristics were often obscured during microscopic analysis due to surface reflectivity, and characteristics were also occasionally obstructed during SEM analysis due to the underlying bone morphology visible with SEM technology. Due to the digital abilities of RTI, neither surface reflectivity nor underlying bone morphology were a difficulty during analysis.

Although great care must be taken when obtaining the photographic sequences necessary for detailed RTI analysis, this study proves that RTI is comparable in accuracy to both microscopic and SEM analysis. RTI is also relatively inexpensive, expeditious, and easy to use in comparison to the more traditional methods. By creating a digital record that can be utilized by experts and untrained observers alike, RTI allows for an accurate, interactive digital record to be shared with other experts or retained for future examination, comparison, and teaching. With further research, RTI has the potential to assist in a holistic order of forensic saw mark analysis, documentation, and presentation.

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**Reflectance Transformation Imaging, Saw Mark Analysis, Forensic Anthropology**



### A48 Micro-Computed Tomography (micro-CT) Analysis of False Starts on Bones After Prolonged Exposure to Fire: An Experimental Study

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**Learning Overview:** After attending this presentation, attendees will be aware of an application of micro-CT for the analysis of false starts on charred bones and its role in the identification of the inflicting tool.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the possible role of microradiology in the field of forensic pathology and anthropology.

**Background:** Recently, micro-CT was successfully applied for the morphologic and morphometric analysis of false starts on bone samples in order to identify the tool used for their production. It is well known that heat-induced transformations of bone tissue include color and dimension variations, fracture development from a macroscopic point of view, and microscopic histological changes.

**Goal:** The purpose of this study is to investigate the role of micro-CT in the identification and characterization of saw marks on human bones before and after exposure to fire, in order to establish if heat-induced changes of the bone may alter false starts.

**Materials and Methods:** A crosscut saw and a ripcut saw were used to produce two different false starts on 30 bone samples obtained from human fibulae. Marks were inflicted manually, sliding the saws and the knives perpendicularly to the long axis of the bone.

The bone samples were divided into two groups: (1) the first group was exposed to the flame of a burner camping stove fire for 3, 9, and 15 minutes; and (2) the second group was exposed to the flame of a burner camping stove for 6, 12, and 18 minutes. Each sample was photographed and scanned using a SkyScan 1172 HR Micro-CT analyzed by micro-CT before and after every period of burning. N-Recon software was utilized for reconstructions, and Digital Imaging and Communications in Medicine (DICOM® Converter® software was used to convert bitmap files in DICOM. OsiriX® open source software, version 7.0.1 was used to elaborate Multiplanar (MPR) reconstructions.

A morphometric assessment was manually performed on the sagittal plane by the same radiologist, measuring the following parameters: top kerf width, depth, bottom kerf width, angles degree, and floor width. The paired student's *t*-test analysis (level of significance set at  $p < 0.01$ ) was used to assess if a statistical significant difference occurred, for each parameter, between false starts produced by the same saw before and after fire exposure.

**Results:** Up to the maximum burning time tested, false starts were macroscopically recognizable after every fire exposure. MicroCT analysis did not show any statistically significant difference in the morphometric features required to identify which saw was used. Micro-CT was able to identify and measure bone marks both on fresh and burned bones. The morphology and number of the detected marks did not change after the burning trial. A minimal reduction of the height and depth was observed after exposure to fire.

**Conclusions:** The fire exposure neither hides the evidence of false starts on bone surface nor invalidates their micro-radiological analysis. MicroCT seems to be a solid technique for the qualitative and quantitative analysis of false starts, even in charred bones.

The preliminary results of this study suggest that micro-CT analysis could be useful for the identification of tool marks on human bones and also after exposure to fire. The possibility of assessing the morphometric features of each lesion indicates that this technique may provide accurate information about the characteristics of the inflicting tool. Although the morphometric analysis of the saw marks also seems feasible after burning, further experiments testing heat-induced changes of the bone at different temperatures and timing of exposure to fire are required to draw any definitive conclusion.

This present study shows that micro-CT is also able to characterize false starts produced by different saws after 18 minutes of exposure to fire. MicroCT seems to be a solid technique for the qualitative and quantitative analysis of false starts, even in charred bones.

#### Forensic Microradiology, False Starts, Charred Bones

### A49 Dehydration-Induced Quantitative Morphometric Alterations to Sharp Force Trauma on Bones

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**Learning Overview:** After attending this presentation, attendees will understand how the taphonomic changes caused by dehydration alter the dimensions of bones as well as the v-shaped cross sections, commonly known as kerf marks, left on bone by sharp-edged objects.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating alterations resulting from a taphonomic agent that is often overlooked in the forensic literature. This presentation will also provide an increased understanding of the reliability of kerf mark data collected from skeletal remains that have been subjected to dehydration.

When human remains are recovered in a forensic context, there is a general understanding that taphonomic processes have affected the presentation of evidence on the body. As human bone is composed of 15%–25% water, dehydration is a taphonomic agent that may cause morphological alterations, including dimensional and volumetric shrinkage and fracture characteristics.<sup>1-3</sup> It has also been suggested that dehydration affects the presentation of striations in sharp force trauma wounds.<sup>4</sup> Despite these findings, dehydration as a taphonomic agent is often overlooked in forensic taphonomy research and forensic analysis.

To quantify the effects of the dehydration process, this study created a controlled experiment that subjected bone samples to lab-induced dehydration. It was hypothesized that the loss of water would affect the morphology of the bones and associated kerf marks, though it was unknown whether these changes would be significant. Domestic pig radii ( $n=9$ ) were macerated and sectioned into samples no wider than 1.5cm ( $n=43$ ). Sharp force trauma was then inflicted on each sample using a household steak knife with a serrated blade. One kerf mark was created on the medial aspect of each section, then immediately photographed to record the pre-dehydration measurements. Bone sections were then subjected to five 24-hour heating and cooling cycles using an Andrew James Food Dehydrator. Samples were weighed using a digital scale after each 24-hour cycle was finished to determine the percentage of water loss. Upon completion of the five heating and cooling cycles, each sample was photographed again to record the post-dehydration measurements. Using ImageJ, six measurements were taken from each sample's before and after photograph: (1) bone section length from the anterior and posterior midpoints; (2) bone section width from the superior and inferior midpoints; (3) bone section medial surface area; (4) kerf mark maximum length; (5) kerf mark maximum width; and (6) kerf mark area. Inter- and intra-observer error tests were completed to ensure consistency in landmark identification and measurements.

Results indicated that both bone sections and kerf marks were altered by the dehydration process. Following the completion of the five 24-hour cycles, the bone samples experienced an average of 22.8% reduction in weight ( $p<.001$ ) and an average of 8.8% reduction in size ( $p<.001$ ). Kerf marks experienced an average size reduction of 28.5% ( $p<.001$ ). This illustrates that kerf mark data obtained from dehydrated skeletal remains may not accurately represent the dimensions of the original wound, thus impacting conclusions regarding type of weapon and trauma infliction in forensic cases. Further, these results demonstrate the demand for future taphonomic research to consider the effects of dehydration on the morphology of bones and bone trauma.

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#### Forensic Anthropology, Taphonomy, Sharp Force Trauma



## A50 Fracture Patterns Associated With an Airplane Propeller Strike: A Case From the West Tennessee Regional Forensic Center

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**Learning Overview:** After attending this presentation, attendees will understand the skeletal trauma patterns that result from propeller strikes and the importance of recognizing and interpreting propeller strike trauma in forensic cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a better understanding of the skeletal trauma patterns that result from propeller strikes in order to improve the ability of anthropologists to recognize this type of trauma in cases in which human remains are recovered from the water and propeller trauma is possible.

Death or injury due to propeller strikes from either a boat or airplane are not common, but they do occur. In 2017, the Coast Guard documented 172 accidents involving a boat propeller, and between 1980 and 2004, the National Transportation Safety Board (NTSB) documented 80 accidents involving airport ground crew struck by airplane propellers.<sup>1,2</sup> Previous studies of propeller trauma due to boat propellers have described severe blunt force injuries, such as longitudinal fractures and delamination to the cranium, and comminuted fractures or complete transection of the long bones.<sup>3,4</sup> Forensic anthropologists may be asked to interpret trauma patterns on remains recovered from the water; thus, it is important for anthropologists to be able to recognize propeller trauma patterns in the skeleton. To better understand propeller trauma patterns in bone, this study examines a case of airplane propeller trauma in a ground crew member who was fatally struck by a moving airplane propeller at the Memphis International Airport in 1998. Using this known case of airplane propeller trauma, this study aims to determine whether there are distinct patterns of skeletal trauma associated with propeller strikes, and if so, to describe and document those patterns.

The bones present consisted of a skull (cranium and mandible), right radius, and right ulna, which were retained at autopsy for anthropological analysis of trauma at the West Tennessee Regional Forensic Center. All elements required reconstruction due to extensive fragmentation. After reconstruction was completed, the trauma type and patterns were described, photographed, and compared to other cases of propeller trauma described in the literature.

The cranium exhibited a longitudinal fracture running transversely across the frontal bone, as well as radiating fractures to the right side of the cranium. An area of delamination was present on the frontal bone. Scalloping and external beveling were present on the frontal bone, and internal beveling was also present at the area of delamination and on the right frontal bone near the temporal line. There was also severe fragmentation of the midfacial region resulting in complete detachment of the maxilla. The right proximal ulna exhibited a comminuted fracture and complete transection at the proximal shaft. The distal right radius also exhibited a comminuted fracture. It was possible to determine at least one impact point on the frontal bone, as well as impacts to the right elbow (proximal ulna) and wrist (distal radius).

The trauma patterns observed in this case (multiple blunt force injuries from multiple high-speed impacts) are consistent with other cases of propeller trauma described in the literature.<sup>3,4</sup> A propeller strike is able to inflict multiple blows in a short period of time resulting in linear longitudinal fractures, delamination, radiating fractures, internal beveling of the cranium, and complete transection or comminuted fractures of the long bones.<sup>4-6</sup> While propeller injuries are uncommon, it is not uncommon for a forensic anthropologist to observe complex trauma patterns and be asked to determine the type and direction of force, as well as the minimum number of impacts. Propeller trauma patterns could mimic other mechanisms of blunt force trauma, thus care should be taken when making interpretations of complex trauma patterns. Given the ability of propellers to inflict multiple high-speed impacts, and the propeller trauma patterns observed in this case study, additional trauma studies that explore uncommon mechanisms of blunt force trauma, such as propellers, are needed.

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### Airplane Propeller Trauma, Skeletal Trauma, Forensic Anthropology



## A51 A Survey of Peri-Mortem Vertebral Trauma in Historic Cases

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**Learning Overview:** After attending this presentation, attendees will be aware of: (1) the value of incorporating vertebrae in trauma analysis; (2) how analysts typically describe and interpret peri-mortem vertebral trauma; and (3) the importance of standardization in descriptions of vertebral skeletal trauma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) facilitating analysis of vertebral trauma in skeletonized remains; (2) highlighting the importance of reconstruction and articulation of fragmented remains for trauma analysis; and (3) improving standardization of vertebral trauma analysis by presenting illustrative examples.

Vertebrae present challenges in trauma analysis because of their unique morphology and articulations within the thorax. Additionally, due to the complex structure of vertebrae, expectations based on biomechanics are of limited value. The forensic anthropological literature presents conflicting opinions regarding the importance and analytical potential of vertebral trauma. Experimental and retrospective trauma research primarily focus on the skull, long bones, and ribs. Several published case studies demonstrate that analysis of vertebral trauma can contribute significantly to overall trauma interpretation. However, in other cases, analysts cite fragmentation of the vertebral remains as precluding any useful interpretative analysis to be undertaken. The foremost objective of this study is to clarify discrepancies in the literature by assessing the value of vertebral trauma analysis in a sample of skeletal trauma analyses of various mechanisms.

Trauma analyses of historic cases associated with United States servicemen and civilians lost during World War II and the Korean War from the Defense POW/MIA Accounting Agency Laboratory are reviewed ( $n=51$ ). Analyses were completed in the blind, and unknown individuals have since been identified and associated with air ( $n=18$ ), ground ( $n=31$ ), and sea losses ( $n=2$ ). These cases display blunt force ( $n=10$ ), projectile ( $n=18$ ), extensive (e.g., deceleration or blast) ( $n=18$ ), or indeterminate ( $n=5$ ) trauma.

Overall, analyses of affected vertebrae are highly variable. The degree of fragmentation is rarely quantified. Most analysts include photographs of vertebral trauma (65%) and incorporate adjacent skeletal regions in their analysis (63%). However, reconstruction or articulation are undertaken in less than half of cases (41%). Vertebral trauma contributes to directionality interpretations in approximately half of cases (51%).

When these results are assessed by mechanism, photographs of vertebral trauma are included more frequently in blunt-force (70%) and projectile (100%) trauma cases than extensive trauma cases (33%). More often than not, analysts incorporate adjacent skeletal regions in vertebral trauma analysis no matter the mechanism (blunt force=60%, projectile=67%, extensive=72%). Vertebrae are reconstructed more commonly in projectile (78%) than blunt force (30%) or extensive (17%) trauma cases, and directionality is interpreted more often in projectile (67%) and extensive (56%) cases than in blunt force (20%) cases. These trends may reflect factors such as the condition (e.g., fragmentation) of vertebrae or the absence of standardized language and guidance for analysis of vertebral skeletal trauma. Additionally, in extensive trauma cases, analysts may not directly address vertebral trauma in an attempt to synthesize observations.

Results indicate that analyses of vertebral trauma are highly variable. Analysts are likely to consider the relationship between trauma observed on vertebrae and adjacent elements regardless of traumatic mechanism. Further, vertebral trauma analysis is underused in blunt force trauma cases, and analysts more often reconstruct vertebrae and infer directionality in projectile trauma cases.

Although vertebrae have received less attention than other elements in the trauma literature, preliminary results suggest that vertebrae are valuable in the interpretation of skeletal trauma regardless of the mechanism of injury. Reconstruction of fragmented vertebral remains and consideration of adjacent elements has the potential to improve analyses and result in more robust descriptions. Though many of the cases reviewed here present relatively complete sets of skeletal remains, these results suggest that vertebrae are valuable in cases with incomplete and fragmented remains as well. These findings facilitate a more comprehensive and standardized interpretation of injury patterns in skeletal remains and direct future research on vertebral trauma in forensic cases.

### Peri-Mortem Trauma Analysis, Vertebral Injury, Mechanism of Trauma



## A52 Prediction of Bullet Type From Cranial Gunshot Trauma

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**Learning Overview:** After attending this presentation, attendees will have learned that it is possible to differentiate bullet type (full metal jacket vs. jacketed hollow point) from measurements of cranial entrance wounds.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the importance of experimental research with gunshot wounds.

Forensic anthropologists have long been interested in cranial gunshot wound interpretation. While being able to understand key extrinsic variables, such as velocity, caliber, distance, type of firearm, and that bullet construction would be useful to a forensic investigation, research has generally fallen short with ways to elucidate such variables from the analysis of bone alone. Retrospective studies that have mined forensic case reports for data have been somewhat useful in terms of establishing patterns, but an experimental approach is superior in its ability to precisely control for extrinsic variables.

Therefore, this project was designed to experimentally test the effect that bullets of the same caliber having two separate constructions (full metal jacket vs. jacketed hollow point) have on cranial gunshot trauma, with the goal being to ascertain whether it would be possible to differentiate between the two different types of bullets from resultant cranial trauma alone. These distinct bullet types are designed to fully penetrate and not fragment (full metal jacket) or penetrate and expand upon contact (jacketed hollow point). Based on the different physical properties of the bullets, it was hypothesized there would be some indication in the cranial trauma that would allow differentiation between the two, with damage from jacketed hollow point bullets being greater.

Forty-five donated adult human heads were obtained from an anatomical tissue supply company, specifically for the purpose of trauma research. A specialized shooting stand was built to support each head at the height of an average adult male, and each head was shot once by the same expert marksman at a distance of three yards either in the frontal bone or parietal/temporal bone, using a revolver with a 1<sup>7</sup>/<sub>8</sub>" barrel loaded with 0.38 caliber bullets. Bullet type (jacketed hollow point vs. full metal jacket) was distributed randomly yet evenly between individuals. Following the experiment, heads were autopsied and macerated using standard procedures.

Entrance wounds, exit wounds (if present), and resultant fractures were examined both endocranially and ectocranially. Three observers independently scored all 45 crania. Quantitative data were collected on the minimum and maximum diameter of the entrance and exit wounds. Qualitative data included evaluation of bevel characteristics, such as its relative size to the wound and whether bevel margins were smooth or crushed, wound shape (i.e., oval, round, or irregular), presence of circumferential delamination around wound margins, and the degree of any cranial fragmentation.

Given missing values from the qualitative data due to not every cranium having an exit wound and to maximize the sample size number of frontal shots ( $n=24$ ) and parietal/temporal shots ( $n=21$ ), only the quantitative data were analyzed here. Logistic regression was used to create a diagnostic test of the quantitative data to include sensitivity (i.e., ability to pick up true positives), specificity (i.e., ability to pick up true negatives), and accuracy; with the jacketed hollow point as the referent bullet. For entrance wounds to the frontal bone, a cut point of 11.0mm minimum diameter had a 90.9% sensitivity, 84.6% specificity, and 87.5% accuracy in determining the bullet type, with the jacketed hollow point entrance more likely to be above 11mm ( $p<.001$ ). For entrance wounds to the parietal/temporal bone, a cut point of 9.5mm minimum diameter had 100% sensitivity, 80% specificity, and a 90.5% accuracy in determining bullet type, with the jacketed hollow point entrance more likely to be above 9.5mm ( $p<.0001$ ).

These results indicate that for .38 caliber bullets, entrance wound diameter at different locations on the cranium is a very strong predictor of bullet construction, with jacketed hollow points creating a larger entrance wound overall. Further research should evaluate if this pattern holds for bullets of other calibers. Finally, these results demonstrate that it is possible to have significant findings with quantitative (and therefore non-subjective) analysis of gunshot wound trauma.

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### Gunshot Wounds, Fracture Analysis, Forensic Anthropology



## A53 Atypical Gunshot Wounds in a Controlled Experiment

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**Learning Overview:** After attending this presentation, attendees will be aware of the variability in cranial gunshot entrance and exit wounds and fracture patterns resulting from a controlled experimental study.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the need for controlled ballistic trauma studies to further elucidate and understand factors that produce variability in cranial gunshot entrance and exit wounds.

The interpretation of gunshot trauma is a key aspect of forensic anthropology, but few studies have systematically tested the effect of gunshot trauma on the cranium. Recently, there has been a push for experimental gunshot studies under controlled conditions in order to expand our knowledge.

This study presents three examples of atypical gunshot fracture patterns that were drawn from a sample of 45 heads belonging to a larger experimental study focusing on gunshot fracture patterns. Prior to shooting, each head was placed atop a six-foot-high shooting stand and held in place using luggage straps. The heads were shot using a Smith and Wesson® model 438 J-frame revolver with a 1<sup>7</sup>/<sub>8</sub>" barrel loaded with 0.38 caliber bullets each weighing 130 grains. Each head was shot once either anteriorly through the frontal bone or laterally through the temporal/parietal bone, using either a Full Metal Jacket (FMJ) bullet or a Jacketed Hollow Point (JHP) bullet. After shooting, the heads were macerated using standard processing techniques. Shot distance and bullet velocity were both controlled for, with each head being shot from three meters away and each bullet fired from the same weapon. Impact velocities ranged from 561f/s to 738f/s, which is considered low velocity. The three examples that follow are fracture patterns that were not expected, given the controlled conditions described above.

**Example 1:** Circumferential delamination around gunshot entrance wounds was observed in both FMJ and JHP bullets. It has been hypothesized that circumferential delamination only occurs in FMJ shots.<sup>1</sup> Presence of circumferential delamination by bullet construction was tested using a Pearson chi-square test with an alpha of .05 and was not significant ( $p=.682$ .) indicating that there is no relationship between the presence of circumferential delamination and bullet construction. These results suggest that the mechanism that causes circumferential delamination is not related to bullet construction. It was hypothesized that circumferential delamination may be related to impact velocity or shot distance, rather than bullet construction.

**Example 2:** A bone plug originating from a frontal entrance wound occurred once during the controlled study. Bone plugs are rarely described in ballistic trauma literature and are an uncommon finding. Additionally, when bone plugs are discovered, they are usually with exit wounds, not entrance wounds. The factors that cause bone plugs are not well understood, and it is thought that velocity plays a large role, with low-velocity gunshots more likely to produce cranial bone plugs. The impact velocity for the shot that produced the entrance wound bone plug was 676f/s, a low-velocity shot; although this was an average velocity for this sample. Further study into what factors of this shot are unique for this particular sample may provide more information about the factors that cause bone plug formation.

**Example 3:** Five of the 45 heads exhibited excessive fracturing or shattering of the cranial vault. For these five heads, the exit wounds did not display any characteristic evidence of normal external beveling. Rather, the observed fracturing appeared to resemble medium- or high-velocity gunshot trauma. Known impact velocities for these shots were average for the study and well within the low-velocity range. Additionally, these five heads had average bone density within the tested sample.

These examples are important to the study of gunshot trauma in forensic anthropology because they demonstrate unexpected results during a controlled experiment. These examples suggest that there is a need for more controlled studies to fully understand the variability of gunshot trauma.

*This project was supported by an award from the National Institute of Justice.*

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### Forensic Anthropology, Gunshot Trauma, Controlled Experiment





## A54 Radiographic Evidence of Gunshot Defects in Skeletal Remains: A Preliminary Study

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**Learning Overview:** The goals of this presentation are to aid in the understanding of how forensic anthropologists utilize radiography for trauma interpretation of gunshot defects and to test the reliability of using radiography in trauma interpretations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by investigating how the decomposition and maceration processes of human remains affect radiographic detection of Radiopaque Material (ROM), which may be useful in trauma interpretation.

Understanding trauma patterns provides forensic anthropologists with necessary contextual information about cause and manner of death. A difficulty in forensic cases for the medical examiner or forensic anthropologist is distinguishing trauma as a Gunshot Wound (GSW) or Blunt Force Trauma (BFT). This difficulty occurs when the remains are incomplete, are highly fragmented, present atypical trauma patterns, or have been exposed to taphonomic processes.<sup>1-4</sup> In some of these cases, ROM (e.g., metallic fragments from the bullet observed on radiographs) can serve as an indicator of a gunshot defect, aiding in trauma interpretation.

While radiographs are commonly used by forensic anthropologists in the detection of opacities to confirm the presence of a GSW, the validity of pursuing opaque material as an indicator of GSW in skeletal remains has not been extensively examined. Specifically, few studies have examined how often opaque materials are observed on radiographs of GSW, and fewer studies have focused on lead particles embedded in the gunshot defects as evidence in cases presenting atypical trauma patterns. The aim of this study is to validate the use of opaque materials detected using radiographs as an indicator of gunshot defects in forensic anthropological investigations involving skeletal remains. This study helps to determine if radiopacities are a reliable indicator of gunshot trauma.

To examine how frequent opacities are present in GSWs, a total of 13 donors from the Texas State Donated Skeletal Collection were radiographed. The donors were radiographed using a portable X-ray machine, or MinX-Ray, at the Osteological Research and Processing Lab at Texas State University. The regions of trauma include cranial, intraoral, and chest and torso. Twelve of these donors were radiographed after the decomposition and maceration processes, resulting in a detection frequency of ROM of 25% (3 donors presented material). One of the donors exhibited material after decomposition, but appeared negative in the radiographs after it was macerated and processed.

Understanding the frequency of ROM detection in GSWs is valuable to the forensic professionals who are involved in trauma interpretation. It is further necessary to understand how the decomposition and maceration processes affect radiographic detection of these materials. This preliminary study exhibits a 25% frequency of ROM detection after both decomposition and maceration, compared to a 33% detection rate only after decomposition. This difference shows that maceration affects detection. Variables that could not be controlled in these real-world cases include shooting range, ammunition type, weapon used, and exposure of remains during the postmortem interval. Further research should include radiographs of GSWs before decomposition, as well as a greater sample and more controlled variables, which may also affect detection but could not be controlled for in this study.

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### Gunshot Defects, Radiography, Decomposition and Maceration



## A55 Fracture Pattern of Midface Ballistic Trauma

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**Learning Overview:** After attending this presentation, attendees will be aware of midface fracture patterns that may assist in differentiating intraoral from submental gunshot wounds.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the research on gunshot wounds to the midface that have direct implications for dry bone analysis.

Gunshot injury signatures (i.e., entrance and exit wound) can often be diagnosed and interpreted at autopsy when soft tissue is present. In the absence of soft tissue, fracture patterns of the hard tissue may be interpreted; however, there is limited information regarding skeletal fracture patterning resulting from intraoral versus submandibular gunshot wounds. In 2005, Fenton et al. reported on five skeletal cases with self-inflicted gunshot wounds to the midline of the skull.<sup>1</sup> They identified a possible diagnostic pattern of bilateral fracturing that occurs as a result of shotgun, rifle, and handgun injuries. The limitation of the 2005 study was that it addressed only five cases, with different firearms: two intraoral rifle, two submandibular or possible submandibular shotgun, and one mid-frontal pistol. The purpose of the current research is to build upon the 2005 study by examining a focal region of the skull; (i.e., the palate; and mandible) of a larger study sample to identify possible distinguishing patterns between intraoral and submandibular gunshot wounds.

This study is a retrospective exploratory investigation of suicides in which a handgun was positioned either intraorally or submentally. The mandible and palate are the skeletal emphases since these are the main points of entry for these types of gunshot wounds. Additionally, this study addresses the relationship of the chambering of the firearm/projectile caliber to the injury pattern. The study sample comprised 187 suicides by intraoral or submental gunshot wounds autopsied from 2010–2019 by the Harris County Institute of Forensic Sciences in Houston, TX. These data were collected by reviewing radiographs, autopsy photographs, and autopsy reports for the presence or absence of a mandibular fracture and/or palate impact. Projectile caliber size was available for all but four cases. The variables investigated were palate impact, the location of the mandibular fracture (anterior, posterior, or both), and the projectile caliber. Logistic regression models were used to identify the relationships between the presence/absence of a mandibular fracture, palate impact, and location of mandibular fractures in relation to the point of entry (submental and intraoral wound). Pearson's chi-square test was used to evaluate the relationship between the presence of mandibular fractures and projectile caliber.

Of the 187 cases, 19 (10%) were submental and 168 (90%) were intraoral. Sixteen of the 19 submental cases (84%) displayed a mandibular fracture, while 31 of the 168 intraoral cases (18%) displayed a mandibular fracture. Of the 19 submental cases, 8 (42%) displayed only an anterior mandibular fracture, zero displayed only a posterior fracture, and 7 (37%) displayed both anterior and posterior fractures. Of the 168 intraoral cases, there were 26 (3.5%) with an anterior fracture, 4 (2.3%) with a posterior mandibular fracture, and 2 with simultaneous anterior and posterior mandibular fractures. Finally, palate impacts were identified in 18 of the 19 submental cases, and 132 of 168 intraoral cases.

The logistic regression model demonstrated that for submental entries, the odds of observing mandibular fractures were significantly greater than not observing mandibular fractures ( $OR=24.04$ , 95% CI [6.53, 88.52];  $p<0.001$ ). In addition, palate impacts were not a significant predictor of point of entry ( $p>0.05$ ). Mandibular fracture location was statistically significant for submental entries ( $p<0.001$ ), and the odds of observing simultaneous anterior and posterior fractures for submental entries was between 10 and 11 times greater than observing anterior and posterior in isolation. Chi-square analysis indicated there was no significant relationship between projectile caliber and the presence of a mandibular fracture ( $p>0.05$ ).

The results of this study are promising for identifying skeletal fracture patterns that may differentiate between intraoral and submental gunshot wounds. One study limitation is the small submental sample size. A larger sample size may provide additional statistical support for interpreting intraoral versus submental gunshot wounds based on mandibular fracture patterns. Future prospective studies—via autopsy and/or Computed Tomography (CT) scanning—should explore the fracture patterning of the entire skull using known cases of intraoral and submental gunshot wounds.

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### Forensic Anthropology, Gunshot Trauma, Trauma Analysis

## A56 The Effects of Diabetes Mellitus (DM) on Bone Fracture Susceptibility and Repair With Applications to Non-Accidental Forensic Fracture Interpretation

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**Learning Overview:** The focus of this research is the examination of the effect of DM upon bone fracture risk and subsequent bone healing. The goals of the study are to identify the bony signatures of DM, determine whether they can be differentiated from other comorbidities affecting the skeleton, and evaluate whether the presence of DM increases fracture risk and alters fracture healing in diabetic individuals.

**Impact on the Forensic Science Community:** This research will impact the forensic science community by providing greater understanding of the influence of DM on bone fracture and healing and lead to the potential for identification of this condition in forensic remains. It will ultimately aid in improving accuracy of forensic trauma interpretations of accidental vs. non-accidental fracture and Time Since Injury (TSI).

Previous research has suggested that diabetes, a chronic metabolic disease characterized by increased blood glucose levels, can result in an array of deleterious effects upon the human skeleton, including irregular distribution of bone mass, decreased bone strength, increased fracture susceptibility, extended healing times, and poor bone regeneration. DM complications and treatments, including hyperglycemia, increased Advanced Glycation End product (AGE) formation, reactive oxygen species generation, inflammation, and the ingestion of insulin and oral antibiotic medicines, can compromise bone health, including its formation, mass, density, distribution, healing process, velocity, and overall healing outcome. DM has been associated with an increase in osteoclastic and thus resorptive bone processes at the expense of osteoblastic (bone forming) ones, although this interpretation has been questioned.<sup>1,2</sup>

However, the specific effects of DM on bone quality and strength, fracture risk, and fracture healing have rarely been studied from an anthropological perspective using known diabetic decedent collections. In the current research, 44 known diabetic decedents, ranging in age from 21 to 84 years, comprise the combined study sample, derived from the University of Tennessee William Bass collection (self-identified as diabetic), the Terry collection (diabetes informed by medical records), and the Radford University Forensic Science Institute (medical records). The majority are affected by Type 2 DM; however, at least two decedents are noted as Type 1. Variables recorded from this sample include presence, type, and location of fracture, character and status of bone healing, and microscopic evidence for bone distribution, density, and overall bone quality (using a 3D digital microscope at 5x–200x magnification).

It is hypothesized that this diabetic sample will manifest a higher fracture frequency and greater number of fractures in non-union or a non-advanced stage of bone repair and remodeling compared to that observed for the non-diabetic decedents in each collection.

Results generally support the stated hypothesis. Fracture frequency is significantly higher (44%) in the diabetic sample, accompanied by earlier (rather than later) fracture healing status for the majority of fractures. At least three decedents manifest amputations in their lower limbs. Microscopic analysis of exposed trabeculae reveals evidence for diminished bone volume in healing fractures, reflected by the presence of smaller bone calluses. Also evident is irregular bone distribution, macroporosity, and overall poor bone quality and strength in the diabetic sample.

However, a confounding limitation of the study was the presence of significant comorbidities associated with diabetic decedents. A majority of the diabetic individuals manifest evidence for osteoporosis, cancers, and other diseases that influence bone quality and complicate attempts to definitively identify bony signatures of DM.

Bone repair for the diabetic sample is interpreted using a model of anabolic compromise in conjunction with catabolic excess and informs the interpretations regarding TSI for fractures observed in diabetic decedents in a forensic context. In conclusion, given the increased fracture risk and fracture healing time variability, as well as decreased bone regenerative capacity for diabetic individuals, it is recommended that caution be exercised in interpretations of non-accidental bone fracture and its TSI in diabetic decedents, particularly for those who are elderly and manifesting associated comorbidities such as osteoporosis.

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### Diabetes Mellitus, Bone Fracture, Bone Healing

## A57 The Reliability of Morphoscopic Data From 3D Surface Scans

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**Learning Overview:** After attending this presentation, attendees will be more knowledgeable of the reliability and accuracy of obtaining morphoscopic data from 3D surface scans, in contrast with traditional methods of data collection utilizing physical human skeletal remains. This presentation will briefly discuss the advantages of 3D surface scans over other technologies, including radiography and Computed Tomography (CT) techniques.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the utility of 3D surface scans in place of physical bones when seeking morphoscopic data and will discuss the potential of 3D surface scans to increase accessibility of human skeletal collections worldwide through the creation of digital skeletal collections, as well as increase sample sizes for future research endeavors. Accordingly, this presentation will contribute to the discussion of the types of data that can be accurately gleaned from such digital skeletal collections.

Digitally formatted human skeletal elements represent an area of recent and increasing interest within forensic anthropology, particularly for the purpose of obtaining metric data.<sup>1,2</sup> Digital human skeletal elements, in the form of CT scans, photogrammetric images, and 3D surface scans, have been used in numerous studies employing geometric morphometric techniques to obtain metric data.<sup>1,2</sup> Research seeking morphoscopic data from similar digitally formatted human skeletal elements, particularly from 3D surface scans, however, is not prevalent within the literature, suggesting that this area has not been thoroughly explored.<sup>3</sup>

3D surface scans of human skeletal elements may be useful in providing morphoscopic data for various parameters of the biological profile, particularly for estimations of sex, age, and ancestry.<sup>4</sup> Previous studies utilizing 3D surface scans and focused on the collection of metric data through geometric morphometrics have reported results of high precision and accuracy.<sup>5-7</sup> Additionally, limited research focused on the collection of non-metric data from 3D representations of bones has also reported findings of high reliability and accuracy.<sup>4,8</sup> Such studies of non-metric data collection, however, have primarily employed a form of CT scan as their data source.<sup>4,9</sup> This study utilizes 3D surface scans as they are more readily produced by forensic anthropologists and others without easy access to clinical medical equipment.<sup>2,3</sup>

This study investigates intra-rater agreement of trait expression utilizing seven non-metric traits of the mandible, including pinching of ascending ramus, shape of inferior border, protrusion of mental eminence, undulation of inferior body, gonial eversion, height of coronoid process, and shape of mandibular notch. These traits were scored twice on a sample of 50 mandibles from the Bass Donated Skeletal Collection, once utilizing digital renderings of the mandibles in the form of 3D surface scans, and a second time using the bones themselves. This sample was comprised of individuals of European and Hispanic descent and included both males and females. 3D scans were synthesized using a NextEngine® 3D laser scanner. Cohen's kappa was used to assess agreement between both sets of scores and all individuals were pooled for statistical analyses, as inter-group differences were not a focus. Standard error and 95% confidence intervals were also calculated for each trait.

The results of these analyses suggest that shape of inferior border, undulation of inferior body, gonial eversion, height of coronoid process, and shape of mandibular notch demonstrate moderate to substantial agreement between 3D surface scans and physical mandibles. These five traits presented kappa values of 0.467 to 0.638 and *p*-values of  $\leq 0.001$ , indicating agreement for these traits was statistically significantly different from zero. Pinching of ascending ramus and protrusion of mental eminence demonstrated smaller levels of agreement. Among all traits included, gonial eversion and shape of mental eminence demonstrated the greatest agreement, with kappa values of 0.524 and 0.638, respectively.

This study serves to demonstrate the potential utility of 3D surface scans in place of physical bones when remains are inaccessible or no longer within an anthropologist's possession. Results demonstrated encouraging results, moderate to substantial agreement between formats, which may become more pronounced in studies of larger sample sizes. Further investigation through future research, as well as investigation of additional skeletal elements, however, is necessary before these techniques should be employed.

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### Morphoscopic, Surface Scan, Inter-Rater Agreement

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## A58 Experimental Assessment of the Surface Quality of 3D Printed Bones for Evaluative Interpretation in Forensic Anthropology Reconstructions

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**Learning Overview:** After attending this presentation, attendees will appreciate the utility of 3D printed replica bones and the challenges around 3D printing fine surface features in forensic anthropology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating challenges associated with the quality and validity of 3D printed bones to accurately portray forensic skeletal material.

The goal of this research was to investigate the surface quality of 3D printed bones, in order to determine if 3D prints can be a suitable proxy to human bones for demonstration of evidence in courts of law. It was expected that the overall shape of the prints would be accurate, but it was unknown whether the quality of the representation of fine surface details would be sufficient.

3D printed replicas of human remains have been shown to be useful tools in courtroom demonstrations of evidence and have been used in several court cases internationally.<sup>1</sup> 3D prints provide a medium that users (such as expert witnesses or jurors) can hold, touch, rotate, and even use to mimic mechanisms of injury.<sup>1,2</sup> Consequently, it is believed that using 3D prints as visual aids may assist laypeople in their understanding of expert/medical testimony.<sup>3</sup> While 3D printed bone replicas have been established as accurate and robust representations of skeletal elements, little research has investigated the representation of fine surface details, such as fracture lines, porosity or texture.<sup>1</sup> Such features are important in the forensic examination of remains, and especially so for exhibiting skeletal trauma in courtrooms.

In this study, nine human bones with differing morphology were digitized using Computed Tomography (CT) scanning on a Toshiba® Aquilion ONE™ Vision Edition scanner. The CT data were reconstructed using 3D Slicer and Blender software following Carew et al. to create 3D models.<sup>1</sup> The 3D models were 3D printed using Selective Laser Sintering (SLS) on an EOSINT® P100 with a white powder material. A three-phased approach was implemented to assess: (1) the metric accuracy of the 3D prints; (2) the viability of applying age and sex estimation methods (with multiple observers;  $n=8$ ); and (3) the surface quality compared to the dry bones, using a customized scoring method (with multiple observers;  $n=8$ ).

The results of phase 1 of this study indicated that the measurement data was reliable. The 3D prints were accurate to within  $\pm 2.0$ mm of the original dry bones, with no statistically significant difference observed between the dry bone and 3D print measurement data ( $p$ -value 0.75).

Phase 2 identified that multiple observers could successfully and confidently perform age and sex estimation methods on the four 3D prints tested when the method utilized gross features. Less success and confidence was seen when these methods utilized fine surface features. The customized ranked qualitative scoring method used in phase 3 found highest scores for surface quality were given for the general morphology of the 3D replicas, followed by detailed morphology, and texture received the lowest scores. Two of the prints (the cranium and mandible) were scored as accurate overall (average total scores  $>11$ ).

This experimental study confirmed the accuracy of the gross morphology of the 3D printed bones, but importantly demonstrated that fine surface details were not always well represented compared to the dry bones. These findings confirm the utility of 3D printed replicas for courtroom exhibition where gross features are required, but suggests caution is necessary for the use of 3D prints when fine detailing is important for evaluative interpretation.

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### 3D Printing, Forensic Anthropology, Evidence Reconstruction



## A59 Investigating the Impact of Opioid Abuse on Intracortical Porosity and Bone Cellular Density: A Synchrotron-Radiation Micro-Computed Tomography (SR $\mu$ CT) Approach

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**Learning Overview:** The goals of this presentation are to: (1) describe how bone tissue pathology associated with opioid use (e.g., increased cortical pore density) is discernable in rib and femur microarchitecture; and (2) present high-resolution SR $\mu$ CT as a tool to characterize opioid-related bone microstructural pathology with a precision that exceeds traditional brightfield microscopy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by disseminating novel research related to the effects of chronic opioid use on bone microstructural integrity. As the opioid crisis is not showing any signs of slowing nationwide, it is critical that further research efforts be undertaken to understand the impacts on bone metabolism. Forensic anthropologists are specifically challenged by the confounding effects of opioid use on age-at-death estimation and fracture assessment from bone tissue.

Prolonged opioid use has been demonstrated to induce osteoporotic-like bone loss and increase the risk of bone fracture in chronic analgesic users. The current theory is that opioids act directly on opioid receptors of bone forming cells (osteoblasts), resulting in more bone resorption than formation. Indirect effects may be caused by lower serum levels of estradiol and testosterone in response to opioids, resulting in decreased bone mineral density and increasing the risk of osteoporosis.<sup>1-3</sup> With the current trend of opioid overdoses poised to expand its impact further, it is critical that additional research related to the impact of chronic opioid use on bone remodeling be undertaken to expand the forensic toolkit. The primary goal of this preliminary study was to describe how opioid use affects microscopic structures and overall porosity of cortical bone microarchitecture at two sites commonly used in histological age-at-death estimation: the mid-shaft sixth rib and anterior femoral mid-shaft. It was hypothesized that: (1) cortical bone porosity in chronic opioid users exceeds that of healthy controls; and (2) the abundance and density of cortical bone's cellular spaces (e.g., osteocyte lacunae) varies between controls and opioid users.

Osteocyte lacunae and cortical porosity were visualized in the anterior femur via SR $\mu$ CT at the Canadian Light Source synchrotron facility in Saskatchewan, Canada. Cortical porosity was visualized in the mid-shaft sixth rib using laboratory micro-CT at The University of Akron's National Polymer Innovation Center. Anterior femoral cores included known opioid users (ages 25, 27) and non-users (ages 21, 90, 93). Mid-shaft sixth ribs were age-matched for known opioid users (ages 24, 25, 27) and non-users (ages 21, 90, 94). Cylindrical Volumes Of Interest (VOIs) were isolated from reconstructed images of cortical bone via Bruker's CT-Analyser image processing software. Osteocyte lacunar spaces were separated from the high-density bone using global thresholding and segmentation. Standard nomenclatures for lacunar indices were applied for the analysis of 3D lacunar parameters within the VOIs. The variables measured included: Total VOI Volume (TV), total Canal Volume within VOI (Ca.V), average Canal Diameter (Ca.Dm), total Number of Lacunae (N.Lc), and average Lacunar Volume (Lc.V). To determine lacunar density per mm<sup>3</sup> (N.Lc/BV), Bone Volume (BV) was calculated as TV-Ca.V. Cortical porosity was calculated as Ca.V/TV.

In the anterior femur, pore density of young drug users (1,606–2,114 pores/mm<sup>3</sup>) considerably exceeded non-users (534–1,444 pores/mm<sup>3</sup>). In the rib, maximum pore density for young drug users (234 pores/mm<sup>3</sup>) similarly exceeded non-users (40–173 pores/mm<sup>3</sup>). Pore thickness in the rib for young drug users (91.7–115.3 $\mu$ m) resembled the young non-users (96.7 $\mu$ m) more than elderly non-users (24.1–24.5 $\mu$ m), as large pores were previously resorbed in the elderly via severe cortical thinning. In the anterior femur, osteocyte lacunar density of young drug users (range=40,213–42,262 lacunae/mm<sup>3</sup>) exceeds both a young non-user (34,834 lacunae/mm<sup>3</sup>) and elderly non-users (range=23,594–29,583 lacunae/mm<sup>3</sup>). These preliminary results suggest that opioid use may accelerate new pore accumulation. This study further detected a concurrent increase in cellular density.

This work represents the first examination of human bone tissue from known opioid users via SR $\mu$ CT. This ongoing research seeks to further characterize opioid use comorbidities such as osteopenia and osteoporosis. Results of this preliminary research have broader applications as they offer promise to identify risk factors for early-onset osteoporotic fracture that have both clinical and forensic applications for predicting and identifying skeletal trauma.

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### Opioid Abuse, 3D Imaging, Cortical Porosity

## A60 Oh Deer! Detecting Non-Human Skeletal Remains Using Bone Collagen Fingerprinting

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**Learning Overview:** The goal of this presentation is to demonstrate through case example the utility of mass spectrometry bone collagen fingerprinting for species identification to distinguish between human and non-human skeletal remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on species identification of unknown bone using proteomic tandem mass spectrometry methods during medicolegal investigations.

A human skeletal case recovered in December 2018 in Parker County, TX, was complicated by the finding of a rib fragment containing 18 Sharp Force Trauma (SFT) kerfs. The remains were partially recovered and commingled with multiple non-human bones, primarily identified, by morphology, as deer (*Odocoileus virginianus*). During the analysis of the skeleton, it was determined that the remains belonged to a middle-aged Hispanic female. Aside from the single rib fragment with SFT, no additional information on the cause of death was observed on the skeleton. As the skeleton was incompletely recovered, it was not possible to immediately rule out the rib fragment as a possible rib of the unidentified female. Observation by the anthropologist suggested that the morphology was not human; however, the pathologist requested additional testing to determine the taxonomic origin of the bone fragment.

To distinguish skeletal remains as human or non-human, professionals in the forensic community use a variety of macroscopic, microscopic, and chemical methods.<sup>1-5</sup> Comparative anatomy differentiates human from non-human bone by examining gross morphological traits, a reflection of skeletal function. Ribs can be problematic to distinguish between humans and non-human mammals of a similar size.<sup>1</sup> Gross morphology can have limited utility when presented with fragmented remains. Histological analysis can reliably distinguish human from non-human bone by observing bone tissue type, primary (plexiform) or secondary (Haversian), as well as other microscopic features of osteon size, Haversian canal size, and density.<sup>2-4</sup> Disadvantages to histomorphometric methods include a need for undecalcified sections, a skill set frequently not available in a medical examiner setting. Additionally, fragmentary or burned remains may not include the exterior layer of bone where plexiform bone, considered diagnostic of non-human bone, is found.<sup>2,3</sup> Chemically, bone collagen is a genetically informative biomolecule. Even after taphonomic processes occur, small amounts of remaining collagen can be obtained and species identified.<sup>5</sup>

The identification of the rib fragment in this case was accomplished by the New York City Office of Chief Medical Examiner (OCME) using a method known as proteomic tandem mass spectrometry to analyze the amino acid sequences of proteins found in bone, soft tissue, and body fluids. Typically, very small bone samples (50mg) are required. In the case of the rib fragment, approximately 5mg of medullary bone was scraped from the rib, and the protein was extracted using 10 volumes of 8 M urea for 72 hours at 4°C and quantified using a bicinchoninic acid protein assay with bovine serum albumin as standard. Twenty µg of sample protein was reduced, alkylated, and digested overnight with trypsin. Peptides were separated by high-performance liquid chromatography using a 30 minute 5%–40% acetonitrile gradient and analyzed by tandem mass spectrometry on a SCIEX™ 6600 TripleTOF. Data were searched against an all-vertebrate National Center for Biotechnology Information (NCBI) database downloaded in April 2018 using SCIEX™ ProteinPilot™ 5 software. Forty-seven proteins were detected with a confidence score ≥95% per a personal communication with Dr. Donald Siegel. The sample submitted to Dr. Siegel at the OCME had a 122 peptide match identifying the rib fragment as *Odocoileus virginianus texanus*, commonly known as a Texas subspecies of the Virginia whitetail deer. The proper identification of this rib fragment with SFT as a deer bone, allowed for the medicolegal investigation to attribute the trauma/modification to an irrelevant behavior.

This case study shows that crossing borders between the fields of forensic anthropology, zooarchaeology, and forensic toxicology has allowed accurate differentiation of non-human skeletal remains of a critical component in a medicolegal investigation. Sharing information and technology across fields of study benefits all, even when each discipline uses the results to answer differing questions.

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### Bone Collagen, Proteomic Mass Spectrometry, Species Identification

## A61 Investigating the Predictive Relationships Between Oxygen and Hydrogen Isotopes in Bottled Water and Human Hair in Mexican Populations

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**Learning Overview:** The goals of this presentation are to: (1) explore the relationship between oxygen ( $\delta^{18}\text{O}$ ) and hydrogen ( $\delta^2\text{H}$ ) in bottled water samples and human hair samples of known Mexican origin; and (2) demonstrate the distribution of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in bottled water samples across the major immigrant-sending states in Mexico to better understand the challenges of using hair as a tool for forensic geolocation in a Mexican context.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting data on the relationship between Mexican bottled water and human hair and the utility of this relationship to act as a predictor of region of origin.

Recently, Juarez et al. reported the application of semi-mechanistic models with adjustable parameters to relate  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in tap water to  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in human hair from Mexico.<sup>1</sup> This study demonstrated that for this modern Mexican sample, positive correlations between isotopes in hair and water were not significant:  $r=0.61$  ( $p=0.05$ ) and  $r=0.60$  ( $p=0.06$ ) for  $^{18}\text{O}$  and  $^2\text{H}$ , respectively. This clearly demonstrated that data from this Mexican population do not exhibit the strong relationships between isotope values of  $^{18}\text{O}$  and  $^2\text{H}$  in tap water and hair characteristic of other populations studied to date. A first order conclusion from this study is that in Mexico, tap water does not equal drinking water. In order to better understand the distribution and variation in  $^{18}\text{O}$  and  $^2\text{H}$  in probable drinking water samples, bottled water samples representing all brand varieties available per sample location (representing 18 total brands) were collected for 16 states in Mexico ( $N=90$  samples). In order to explore the relationship between bottled water and hair samples, this study used a subset of paired samples representing 8 states with  $N=62$  hair samples and  $N=70$  bottled water samples.

The results are reported here using delta notation and the Vienna Standard Mean Ocean Water (VSMOW) scale. General statistics were analyzed using SPSS version 25.0. Bottled water values spanned a range from +0.5‰ to -11.5‰ and +1.3‰ to -80.2‰ for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ , respectively. The lowest sample values came from Tepic Nayarit, but similarly low values were also found in Michoacán, Mexico City, Morelos, Oaxaca, and Campeche. The highest sample values were found in samples from Campeche and Tabasco. Isotope levels in bottled water samples differed in their standard deviation by collection location, ranging from 7.3‰ to 0.03‰ for  $\delta^{18}\text{O}$  and 48.6‰ to 0.3‰ for  $\delta^2\text{H}$ . Samples from Nayarit showed the highest level of deviation. Isotope values in hair ranged from +9.5‰ to +16.1‰ and -90.8‰ to -53.7‰ for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ , respectively. The most depleted  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  hair values came from individuals in the state of Morelos.

Linear regression for the paired hair and bottled water samples were significant for H isotopes but not for O isotopes ( $\delta^2\text{H} = -18.09 + 0.828 * \delta^2\text{Hw}$ ,  $R^2=0.7$ ,  $R=0.84$ ,  $p=0.01$ ;  $R^2=0.43$ ,  $R=0.2$ ,  $p=0.6$ ). Correlation coefficients for statistically significant relationships were lower than those previously published for tap water/hair or precipitation/hair relationships from other regions.

The variation in bottled water samples by state suggests that multiple sources for bottled water exist for purchase in each locality. While bottled water has comparatively better fits to hair data by state than previously published tap water data, the inconsistent results between this study and those on other regions suggests shifting water usage. Mexico has the highest consumption of bottled water per capita in the world. This study clearly demonstrates that in order to use water isotopes in Mexican hair to determine region of origin, these cultural contexts must be taken into consideration, and a better understanding of bottled water variation and usage is needed.

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### Bottled Water, Hair, Mexico





## A62 An Assessment of How “Predictive” Human Geolocation Models Perform When Compared to a “Known” Geolocated Human Enamel Data-Set

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**Learning Overview:** After attending this presentation, attendees will understand the forensic importance of analyzing oxygen stable isotopes in human enamel ( $\delta^{18}\text{O}_c$ ) for the utilization of stable isotope analysis for human geolocation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding to the existing knowledge of the relationship between  $\delta^{18}\text{O}_c$  values and that of drinking water ( $\delta^{18}\text{O}_{dw}$ ) by presenting  $\delta^{18}\text{O}_c$  values in modern human dental enamel with known provenance.

The geographical life history of an individual can be determined by analyzing stable isotopes in human tissues. The isotopic compositions of tissues are reflective of the environment during tissue formation, with drinking water ( $\delta^{18}\text{O}_{dw}$ ) contributing significantly to  $\delta^{18}\text{O}_c$ . The current approach to human provenancing utilizes available tap or drinking water data as the base for placing individuals on a geographical map. This is believed to be possible through the understanding of the relationship between  $\delta^{18}\text{O}_c$  and  $\delta^{18}\text{O}_{dw}$ . However, limited data is available for known modern  $\delta^{18}\text{O}_c$ , and no known data exist for any Canadian city.

This study investigated the isotopic composition of oxygen stable isotopes in known modern human enamel collected from across Metro Vancouver (MV) and British Columbia, Canada, and tested the relationship between  $\delta^{18}\text{O}_c$  and  $\delta^{18}\text{O}$  of tap water ( $\delta^{18}\text{O}_{tap}$ ). Isotopic data were compared against known MV tap water values. Results were also compared against the range of  $\delta^{18}\text{O}_c$  values predicted from  $\delta^{18}\text{O}_{tap}$  values with linear models reported by other published studies. A total of 41 extracted human molar samples were collected from dental clinics across MV during the years 2015 to 2017. Information on location of residence and any relocations from birth to 25 years was also retrieved from each individual. This was to ensure that known geographical information was acquired from the individuals during the time of tissue formation. Enamel was extracted into powder form (~1mg) by gentle abrasion with a Dremel® diamond-tipped, hand-held drill. Samples were subsequently analyzed on a Delta® Plus XP isotope ratio mass spectrometer with an analytical precision of  $\pm 0.2\text{‰}$  for  $\delta^{18}\text{O}$ .

Twenty-four of the 41 individuals were of MV origin, two with mixed Canadian origins, and the remaining individuals were from various cities across Canada. The results revealed that  $\delta^{18}\text{O}_c$  values in tooth enamel from individuals residing in MV during the time of tissue formation showed a range of 3.8‰ with a mean of  $-8.7\text{‰} \pm 0.8$  ( $n=24$ ). Mean  $\delta^{18}\text{O}_c$  for all Canadian values were  $-8.7\text{‰} \pm 1.1$  ( $n=41$ ) with a range of 5.7‰.

The results from this study indicate there is a positive but weak ( $p>0.05$ ) correlation between  $\delta^{18}\text{O}$  values in known modern human enamel and  $\delta^{18}\text{O}$  values in known source tap water. Furthermore, the range of  $\delta^{18}\text{O}_c$  values observed for a single city of MV is greater than the range for an entire country such as Bulgaria, Japan, or the United Kingdom. The wide range in  $\delta^{18}\text{O}_c$  values from modern MV residents may be due to the contribution of multiple water sources for MV tap water, which also demonstrates the importance of establishing the isotopic range for individual cities beyond this study. Furthermore, up to 70% of MV individuals were identified as residing outside of MV when utilizing predictive models from other published studies. This finding has significant forensic importance as it demonstrates the weakness of current predictive equations. It is concluded that the theoretical isotopic relationship between human enamel formation and drinking water is valid, and that predictive models need to be built on a solid foundation of known samples.

### Stable Isotopes, Enamel, Human Geolocation

### A63 Subadult Body Mass Estimation From Skeletal Remains: Validation for Femoral Cross-Section Methods in a Contemporary Taiwanese Population

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the validity of body mass estimation equations for subadult skeletons using femoral cross-section and whether it is suitable to apply such equations in different populations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing population data from an underrepresented area of the world and will promote better understanding of how cultural and ecological contexts affect subadult body mass estimation.

Body mass estimation for subadult skeletons is sometimes required in forensic anthropological analysis and can provide important and useful information in subsequent legal investigations. Several methods are available for estimating subadult body mass from skeletons, either using external measurements or cross-sectional measures of the femur. Specifically, past studies suggested that the two methods using femoral cross-section: age-structured equations developed by Robbins et al. and a panel regression-based equation developed by Robbins Schug et al. may yield more accurate results compared to equations using external measurements of the femur.<sup>1,2</sup> However, these methods have not been tested in contemporary samples of Asian descent, and their validity in these populations is largely unknown.

This study tested both the age-structured equations and the panel regression-derived equation for body mass estimation in a contemporary Taiwanese population. Computed Tomography (CT) scans of individuals aged 0 to 16 years of both sexes were collected from the National Taiwan University Hospital (NTUH). Individuals with fractured femur or visible anomalies were excluded. Cross-sectional properties were measured at approximately 50% length. Estimated body mass was compared to documented body mass to evaluate the performance of the two methods.

The results indicate that both methods performed relatively similarly in a contemporary Taiwanese population. The accuracy (absolute mean differences) for age-structured equations is 13.99kg while the accuracy from the panel regression equation is 19.64kg. The bias (mean differences) for age-structured equations is -6.83kg, while the bias for panel regression equation is 19.15kg. These values far exceed those observed previously in populations of mostly European and African descent. In general, the age-structured method tends to overestimate body mass while the panel regression method tends to underestimate body mass in the Taiwanese population. Moreover, Root Mean Squared Error (RMSE) was calculated for different age categories to investigate whether age has an effect on the estimation. Results show that as age category increases, deviation of estimated body mass from true body mass increases for the panel regression-derived equation, while the reverse is true for age-structured equations. Suggestions are made in light of these findings and statistical procedures are developed for future references.

While hospital samples may not be the best representation of a population, this study highlights the need to test the validity of methods for body mass estimation for subadult skeletons in different populations, especially in underrepresented areas of the world.

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#### Body Mass, Subadult, Forensic Anthropology

## A64 A Comparative Analysis of Nasal Profile Estimation Methods for Facial Reconstruction

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**Learning Overview:** After attending this presentation, attendees will possess information of methods to investigate the nasal profile for enhancing the accuracy of facial reconstruction. In addition, attendees will better understand the importance of basal research for population-specific facial reconstruction.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing comparative research of the nasal profile for enhancing the accuracy of facial reconstruction.

The nose is an important facial feature that significantly affects the outcome of facial reconstruction and its recognition. For the 3D measurement analysis, the skin and bone of the nasal area were remodeled utilizing Computed Tomography (CT) images. Then the methods and results of previous facial reconstruction studies on the nasal aperture area with Korean adult samples were compared. One hundred CT scans of Korean adults were selected for this study. The subjects were patients who undertook craniofacial CT scans for clinical purposes at the Department of Radiology at St. Mary's Hospital in Seoul, South Korea. Patients with a history of head trauma, severe deformities, or asymmetry were excluded from this study. 3D models of the cranium and face were remodeled and measured by Mimics® software, version 14.1. Six studies were selected to compare various sample populations and materials for nasal profile reconstruction.<sup>1-6</sup>

The measurement system for 3D models was programmed with referring landmarks from previous studies to facilitate comparison process. In cases of referring measurements and landmarks of lateral cephalograms, the landmarks were positioned at the profile view of each 3D model. The paired *t*-test of corresponding measurement with previous studies showed there was a significant difference between actually measured values that are measured in the same manner as previous studies and predicted values that are calculated from regression equations of previous studies ( $p < 0.05$ ). As the results have shown, differences of research material, of whether to use cephalogram or 3D models, and a measurement method that involves 2D measurement and 3D measurement was an important factor in deciding its accuracy for nasal profile reconstruction. Moreover ethnic-specific differences in the field of facial reconstruction should be taken into considerable consideration since studies made quite different predictions.

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### Nasal Profile, Comparative Analysis, Facial Reconstruction

### A65 The Application of Morphometric and Morphoscopic Features of the Nose in Facial Reconstruction: A Study on a North Indian Population

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**Learning Overview:** After attending this presentation, attendees will be better informed about metric and non-metric traits of the human nose, which may help to identify a particular population group or an individual.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new dataset on noses that will help in facial reconstruction and sex determination in forensic casework.

Forensic facial reconstruction is an ancillary method used to identify the dead. The goal of forensic facial reconstruction is to recreate the face of the deceased with the help of anthropological analysis of the skull followed by facial reconstruction. The face of the person has several different types of exclusionary features that are of great importance in the identification and recognition of a person. The nose is considered to be one of the conspicuous features for facial approximations during the recognition process. Therefore, subtle changes in nasal morphology may lead to considerable differences in the appearance and face characteristics of an individual. In cases of mass disasters, such as earthquakes, terrorists attacks, road accidents, and landslides, mutilated parts of the bodies are brought for identification. When part of the face is disfigured, ethnic-specific models can be of great help in reconstructing the face. In forensic facial reconstruction, both metric and non-metric data serve as the basis of reliable approximations by comparing antemortem and postmortem data.

The current study aims to provide a wide range of morphometric and morphoscopic data on the nose. The study was conducted on a total of 508 subjects (264 males and 244 females) on two ethnic populations, Rajputs and Brahmins (Highland Himalayan population), of the Himachal Pradesh State in North India. Morphometric variables of the nose include nasal height, nasal breadth, nasal depth, anatomical width of the nose, and total length of the nasal bridge; morphoscopic traits include nose size, nasal bridge profile, nasal root shape, tip of the nose, its projection, alare profile, nasal septum features, and the nasal cavity. Each measurement was taken with the help of a sliding caliper. The action was performed on all subjects by the same examiner. For non-metric traits, the subjects were first photographed from basal, lateral, and frontal nose view by a Sony Cyber-shot™ DSCW80 7.2MP digital camera, then studied. The results of the statistical analysis (descriptive) performed using SPSS reveal that mean values of nasal and facial parameters are larger for males. *T*-test and chi-square statistics confirm the significant sexual dimorphism in metric and non-metric variables, respectively. To keep this in mind, Binary Logistic Regression statistics were applied to formulate models for sex determination from these variables. The overall sex predictive accuracy from nose variables were 78.1%. For nose reconstruction from facial variables, regression models were prepared for each sex separately. The correlation coefficient for morphological and physiognomic facial length with nasal length was 0.539 and 0.382 for males; 0.349 and 0.251 for females, respectively.

Highland Himalayan populations are more prone to natural disasters resulting in mass deaths. In such cases, reconstruction of the nose from the remaining face can boost the identification process. The present study confirms the sexual dimorphism in facial features and forms. Robust statistical procedures were adopted for reliable construction of the nose from facial measurements.

#### Forensic Anthropology, Facial Reconstruction, Identification

## A66 Nose Approximation From Cone-Beam Computed Tomography (CBCT) Using a New Computer-Assisted Method

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**Learning Overview:** After attending this presentation, attendees will have gained knowledge on an automated landmarking method for the nasal substrate and the production of accurate statistical models, which are optimized with the inclusion of ancestry, sex, and age, for estimating nasal shape among two South African groups. Automatic landmarking, in addition to reducing errors, achieved better precision for Facial Approximation (FA), enabling a convenient prerequisite for geometric morphometrics and the future possibility of including more samples and populations with ease.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing a novel 3D approach to FA methods in South Africa. Recent developments in computer science and advances in medical imaging techniques, such as surface, Magnetic Resonance Imaging (MRI)-, Computed Tomography (CT)-, and CBCT-scanning resulted in large databases of 3D representations of hard- and soft-tissues of the crania of living people. Researchers in the field of FA can utilize these technologies to develop alternative computer-based techniques to improve objectivity of approximations for both criminal investigations and unidentified persons.<sup>1,2</sup>

South Africa is in a humanitarian crisis with vast numbers of unidentified persons buried in paupers' graves or incinerated each year. Because of socio-political and historical circumstances, the unidentified are often from impoverished communities with reduced access to medical and police services. Creative approaches are therefore required to improve identification of decomposed and skeletonized remains. FA techniques are often employed in these situations to communicate with the public, as the face is often the sole means from which a person is remembered and recognized. Main critiques of FA methods are inherent subjectivity, lack of standardization, few reference samples, and poor correlations between hard and soft tissues. In light of the great demand for the identification of unknown remains in South Africa, a need exists to establish reliable FA techniques that will not only take into account sex and age, but most importantly be specific for South Africans. The purpose of this study was to provide an automated computer-assisted method to create accurate statistical models for predicting nasal soft tissue from information about the underlying skull substrate using CBCT scans.

A total of 200 CBCT scans of 100 Black (67 male, 33 female) and 100 (35 male, 65 female) White South Africans were selected from the Oral and Dental Hospital, University of Pretoria, and the Life Groenkloof Hospital, Pretoria, South Africa. The acquisition and extraction of the 3D-relevant anatomical structures (hard and soft tissue) was performed by an automated 3D method based on an automatic dense landmarking procedure using MeVisLab® v. 2.7.1 software.<sup>3</sup> An evaluation of shape differences attributed to known factors (ancestry, sex, size, and age) were performed using geometric morphometrics, and statistical models of prediction were created using a Projection onto Latent Structures Regression (PLSR) algorithm. The accuracy of estimating soft tissue of the nose was evaluated in terms of metric deviations on training and on untrained datasets. Variability in the hard and soft tissue of the nose was noted for sex, age, and allometry among the two population groups. When using the landmark-to-landmark distances, prediction errors ranged between 1.769mm and 2.164mm for Black South Africans at the tip of the nose and the alae, and between 2.068mm to 2.175mm for White South Africans. The prediction errors on untrained data were slightly larger, ranging between 2.139mm and 2.833mm for the Black South Africans and 2.575mm to 2.859mm for White South Africans.

Global advances in 3D research into human variation and skeletal anatomy will improve creation of biological profiles and FA methods, which will be of particular relevance to identify unknown persons in disadvantaged groups in Africa. This study demonstrated the utilization of an automated 3D method as a convenient prerequisite for providing a valid and reliable nose prediction model independent of any artistic interpretations.

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### Statistical Models, Shape Variation, Automatic Landmarking



### A67 The First Recovery and Identification Project of Missing Casualties in the Korean Demilitarized Zone (DMZ)

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**Learning Overview:** After attending this presentation, attendees will understand some principles of an unprecedented recovery and identification project of casualties from the Korean DMZ.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a key aspect for identifying the missing personnel recovered from an inaccessible area for a long period of time.

The Korean Peninsula has been divided into South and North Korea since the three-year Korean War ended in 1953. This peninsula is the last remaining divided territory within the same nation in the world. The Korean DMZ was established after the Korean Armistice Agreement was signed in 1953. Since then, for nearly 70 years, it has functioned as a militarized buffer zone between South and North Korea and has become a unique area untouched by humans for military reasons. After two Inter-Korean Summits (in April and September 2018), the Korean government commenced the recovery of war remains in the DMZ in October of 2018).

Based on the war's history, at the main recovery area in the DMZ, Arrowhead Ridge, Korean and United Nations forces fought against Chinese troops, resulting in more than 300 soldiers from South Korea, the United States, and France being killed. At first, both sides agreed to make a South-North Korea Joint Recovery Team. However, as the political situation became complicated, only the Ministry of National Defense Agency of KIA Recovery & Identification (MAKRI) is conducting recovery efforts in the DMZ. Before the excavation, land mines and other explosives were removed in the recovery area for safety reasons. At this point, more than 100 missing personnel have been exhumed and transported to the MAKRI Central Identification Laboratory (CIL) for further analysis. Remains recovered from the DMZ were probably all male, usually in their late teens to mid-twenties. One of the recovered was identified and returned to his bereaved family. In addition, one set of remains presumed to be a United Nations soldier (possibly from American or French troops) was recovered. Due to the fact that the DMZ has been kept intact, conditions of the recovered human remains and personal belongings are relatively suitable and the burial sites well preserved.

This study aims to examine human skeletal remains that were unexpectedly protected from external environmental changes, such as industrialization. For this purpose, burial context, ancestry, trauma, and pathological changes were assessed. This report will present valuable information concerning body decomposition and weathering processes after death. It is recommended that forensic specialists pay special attention to the Korean DMZ recovery and identification project.

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**Korean DMZ, Korean War, South-North Korea Joint Team**



### A68 Historical and Cultural Complications of Repatriating Forensic “Cases” in the Southeastern United States

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**Learning Overview:** After attending this presentation, attendees will understand some of the challenges facing the identification of historic Native American remains in the southeastern United States that have been submitted as forensic cases to a medical examiner’s office.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showcasing how a historical and cultural understanding of a region is vital to the appropriate classification of historic Native American remains in a medicolegal setting.

The Georgia Bureau of Investigation (GBI) houses the skeletal remains of unidentified individuals ( $N=272$ ) dating back to the 1960s. The GBI serves 153 of the 159 counties in the state, resulting in a wide and varied collection of remains that have since become cold cases. Legislation was passed in 2016 to allow for the burial of these cases. Prior to interment, it was decided that a thorough examination of every case was necessary to create a robust case file in the event that a lead on identification arises. Of these cold cases, anthropological analyses were used to determine that a small number ( $n=7$ ) were individuals of Native American descent whose deaths likely occurred before the present day. Each set of remains was photographed, inventoried, and analyzed using gross morphological techniques. Craniometric data was collected using a MicroScribe® digitizer and 3Skull analytical software. Each individual was compared to the Forensic Databank and Howell’s Dataset using FORDISC® 3.1. Four individuals were classified as “American Indian” with an F-typicality greater than 0.7. The remaining three individuals could not be reliably grouped with those in either dataset.

In addition to craniometrics analyses, the identification of historic Native remains in a forensic context requires a knowledge of the geomorphology and taphonomy unique to the various regions of Georgia. Georgia has a large piedmont region, coastal plain, and ridge and valley system. These taphonomic signatures, including red clay staining and increased friability from highly acidic soils, have yet to be documented in a medicolegal context.

Further, it is argued through this study that the historical and cultural environment in Georgia has increased the difficulty in identifying and repatriating these types of remains. While Georgia was one of the most highly occupied regions in the United States during the Mississippian Period (AD 800–1600), native Georgia Indians are not currently recognized as independent nations. In 1992, the Georgia Council on American Indian Concerns was created to mitigate and protect Native sites and help repatriate remains. The Council serves as the governing body for decisions concerning Native burials found in archaeological contexts; however, Native remains recovered in a medicolegal investigation are rarely brought to the attention of the Council.

In response to these issues, the GBI Office of the Medical Examiner has made it a priority to work in concert with the Native Council and develop a plan for transferring custody of these remains and establishing a concrete pipeline for future “cases.” Further, an educational program has been implemented to teach local law enforcement officers about the likelihood of recovering Native remains and appropriate protocols for reporting.

While all cases in the GBI’s unidentified collection will receive a thorough examination, it is suggested that the taphonomic signatures of this region are unique and should be documented to decrease misclassification of Native remains and expedite their repatriation through available channels.

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#### Unidentified, Native American, Craniometrics



### A69 Skeletal Preparation Using a Modified Steam Kettle: A Fast, Easy, and Low-Maintenance Method for Processing Human Remains

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**Learning Overview:** After attending this presentation, attendees will understand the process of utilizing a steam kettle for skeletal preparation, including strategies that improve the final quality of skeletal remains and decrease overall handling and processing time, and will recognize the benefits of utilizing a modified steam kettle for skeletal preparation of human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a method for soft tissue removal developed at Western Michigan University Homer Stryker M.D. School of Medicine that improves the efficiency of processing human skeletal remains while decreasing the opportunity for damage to the skeletal remains.

The removal of soft tissue without damage to bone is an integral part of forensic anthropology, specifically in the analysis of skeletal anatomy and trauma. Forensic anthropologists assist with reconstruction of the biological profile, identification, and trauma assessment when examination of the soft tissue is no longer possible due to decomposition, thermal damage, or trauma. Typically, scalpels and other metal implements are used in the removal of soft tissues and disarticulation of the fleshed decedent before maceration. Crock-pots or pots heated over Bunsen burners are often used for warm water maceration with chemical agents, such as sodium carbonate, other detergents, ammonia, chlorine, and dish soaps to break down the proteins and fats. This process requires extensive disarticulation of the remains into small body segments to fit them in small maceration vessels. This prolongs the duration of disarticulation and increases the likelihood of creating processing artifacts such as cut marks to the skeletal elements and joints. Furthermore, if maceration is not appropriately monitored, burning of the skeletal material can occur, irreparably damaging the elements and potentially obscuring pre-existing trauma. Using a steam kettle with a basket insert allows large body segments, such as intact extremities and articulated head-torso-pelvis segments, to be placed directly on an elevated basket in the steam kettle. This method demonstrates the ability to easily macerate skeletal remains without the use of chemicals, minimizing the risk of damaging bone, and greatly reducing the amount of hands-on time spent disarticulating and subsequently cleaning the bones.

Study materials include 18 donors of the Western Michigan University Homer Stryker M.D. School of Medicine Body Donation Program who elected to be included in the Skeletal Research and Teaching Collection. The water temperature, steam kettle settings, and duration of maceration were recorded throughout each skeletal processing and were varied between donors to understand the impact on the quality of the skeletal remains. In addition, the length and type (cooler vs. freezer) of storage and the condition of the decedent were recorded to explore the impact of storage temperatures and durations on bone quality. Donors were macerated at water temperatures that varied between 40°C and 94°C. The duration of maceration varied between two and nine days. The conditions that produced the best bone quality were a maceration temperature of 90°C, with no temperature fluctuation, for a duration of two days.

The preparation of skeletal material for analysis by the removal of soft tissue is an important part of skeletal analysis in forensic anthropology. Forensic anthropologists should be cognizant of the advantages and disadvantages of the different methods of skeletal preparation in order to utilize the method that best suits their needs and decreases the likelihood of damaging bone. This method produces skeletal elements that are in excellent condition for analysis. This new approach to skeletal preparation will improve the efficiency and final disposition of the skeletal remains.

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**Forensic Anthropology, Skeletal Preparation, Forensic Science**





## A70 Salary, Wage Transparency, and Forensic Anthropology

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**Learning Overview:** After attending this presentation, attendees will understand the current distribution of salaries of forensic anthropologists in the United States as well as the primary factors that affect forensic anthropologists' salaries.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about the salaries of practicing forensic anthropologists in the United States within the context of the need for increased wage transparency and salary equity.

Forensic anthropology, like all forensic sciences, is a discipline meant to serve the public. As such, nearly all forensic anthropologists in the United States are employed as public servants, with individuals being employed by government-funded institutions either at the state or federal level. In the United States, the salaries of government employees are considered to be public information and because of this, these salaries are often freely available in publicly accessible databases or via requests for information using such avenues as the Freedom of Information Act (FOIA).

The reason salary information is important is because salary transparency narrows wage disparities, reduces favoritism and discrimination, increases the bargaining power of employees, and potentially causes employers to focus more on salary differentiation in terms of productivity and seniority; essentially wage transparency generates greater equity among employees.<sup>1</sup> Estlund states: "accurate information about other people's salaries in one's own and other organizations is crucial to decisions about seeking a better opportunity elsewhere, asking for a raise, or simply staying on the job and accepting the status quo."<sup>1</sup> Employers currently benefit from a lack of transparency in salaries through asymmetrical information, as there is no largely accessible data on salaries in forensic anthropology, within academia or applied institutions.

In order to examine the salaries of forensic anthropologists in the United States, salary information for current members of the anthropology section of the American Academy of Forensic Sciences (AAFS) were searched for on freely accessible public internet databases. In addition to this data, the following information was also examined for each individual: sex, terminal degree, certification by the American Board of Forensic Anthropology (ABFA), type of position (academic vs. applied), academic institution classification (using Carnegie Classifications), and rank of employee (e.g., assistant professor, director, etc.).

Results found that salary information was available for 120 individuals ( $n=78$  females and  $n=42$  males) from various academic ( $n=82$ ) and applied ( $n=38$ ) institutions within the United States. For academic individuals, most had traditional ranks ( $n=15$  term positions,  $n=27$  assistant professors,  $n=15$  associate professors, and  $n=21$  full professors), although a number had additional administrative duties ( $n=16$ ). In order to standardize individual salaries, each salary was divided by the composite cost of living index for each state (from the Missouri Department of Economic Development), followed by the term length of each individual (creating a "Salary Index"). Salary Index was compared within variable groups using Kruskal-Wallis rank sum tests, and Analysis of Variance (ANOVA) was used to compare Salary Index between groups. There was a statistically significant ( $p<0.01$ ) difference between mean Salary Index of Academic and Applied forensic anthropologists, and two sub-groups using rank of employee were used for further analysis. Linear regression equations were created using available data as having independent and interactional effects to estimate Salary Index for Academic ( $R^2=0.7268$ ) and Applied ( $R^2=0.07486$ ) forensic anthropologists. Even with a strong  $R^2$  value for the Academic sub-group, a high residual sum of square value demonstrate this model has limited ability for Salary Index estimation.

The results of this research demonstrate inconsistencies in pay for forensic anthropologists, especially for those working in the Applied sector. Variables such as basic classification of institution and rank of employee were statistically significant factors ( $p < 0.001$ ) that affected Salary Index of Academic forensic anthropologists. Additional information such as time in a position/at an institution may provide insightful information as to Salary Index for future research. Finally, wage transparency paired with increased standardization into the qualifications of forensic anthropologists may assist in future wage equity in the discipline.

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### Salary, Wage Transparency, Professionalism



### A71 Learning From Our Casework: The Forensic Anthropology Database for Assessing Methods Accuracy (FADAMA)

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**Learning Overview:** After attending this presentation, attendees will understand how data from the FADAMA is informing research on methods use and accuracy, how other researchers can use and access the data, as well as the new data and platform additions to the database in the past two years.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing practitioners on how FADAMA supports a vital aspect of the discipline's ability to self-assess its progress and success in terms of casework outcomes.

In 2013, the development of the database was initiated by the Society of Forensic Anthropologists in order to provide a comprehensive and centralized open-source database on methods use and accuracy in forensic anthropology casework. Previously, there existed no formal, organized space for the forensic anthropology community to share approaches to casework and casework outcomes (e.g., method-derived versus actual biological profile information).

This presentation will introduce user platform additions to FADAMA as well as new collaborative opportunities and strategies for data collection support. Additions to the user platform for the database include data on method-specific outcomes, which allows for the assessment of method-specific accuracy. Beginning in the spring of 2020, a trained technician will be available to travel to interested laboratories to assist with data input, alleviating time constraints on participating facilities.

Furthermore, this presentation will address the following research questions with the current data included in the database: (1) accuracy of practitioner-generated estimates of the biological profile, (2) the trends in method use, and (3) decedent demographic trends.

As of August 1, 2019, a total of 243 cases representing a wide range of case data are present in FADAMA, with continued commitments from several high-caseload offices to submit their cases, such as the University of North Texas Center for Human Identification and the New York City Office of Chief Medical Examiner. The current reported case demographics are as follows: Case Year (range=1981-2018,  $\mu=2009.7$ ), Sex ( $n_{\text{female}}=73$ ,  $n_{\text{male}}=169$ ), Age (range=14-94 years,  $\mu=42.6$ ), Ancestry ( $n_{\text{Asian}}=14$ ,  $n_{\text{black}}=65$ ,  $n_{\text{hispanic}}=39$ ,  $n_{\text{other}}=2$ ,  $n_{\text{white}}=107$ ), and Stature (range=48-77 inches,  $\mu_{\text{females}}=63.5$  inches,  $\mu_{\text{males}}=69.5$  inches). Trends in biological profile estimation accuracies are as follows: Age=98%, Stature=90%, Sex=99.9%, Ancestry=92%. Additionally, more than 30 forensic anthropological methods were reported by practitioners to assess the biological profile. This presentation will review trends in method use for age, sex, ancestry, and stature, as well as biological profile accuracy statistics for the submitted cases. Furthermore, this presentation will review the submission process, including case eligibility, appropriate case data, and permissions and responsibilities of FADAMA users.

As case data continues to accumulate, interested forensic anthropologists/researchers can access and analyze the anonymized case data (electronically available for download at: <http://sofainc.org/sofadb/index.php>). This presentation will include a live demonstration of the database for interested attendees.

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#### Database, Case Reports, Accuracy

## A72 3D Slicer as a Tool for Creating Virtual Bone Models From Computed Tomography (CT) Data: Toward Validating a Step-By-Step Method

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**Learning Overview:** The goal of this presentation is to provide insight into how the step-by-step method developed in this study can increase availability of 3D models that are consistent, reproducible, and accurate for application in forensic anthropology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a method that can improve a user's ability to handle CT scan data and develop 3D modeling performance.

This research sought to create an easy-to-use, step-by-step method for modeling CT data and to validate its application to the production of 3D models through testing the robustness of 3D models produced by first-time and experienced users.

CT scans are becoming more widely available and are offering significant opportunities for both traditional and novel methods of identification in forensic anthropology. At the moment, there are a wide range of software packages designed to build 3D models using CT data; however, these have not been validated or tested for their reproducibility in forensic anthropology. Therefore, this study sought to develop a method using open source (free) software to establish a valid technique for producing reliable, accurate, and reproducible 3D models from CT scan data.

A step-by-step method was developed for creating 3D models using 3D Slicer, a free open-source CT reconstruction software. The five observers selected for this study included three individuals with no prior experience in handling or producing 3D models and two with limited experience. The observers tested this method by producing 3D crania models from 20 clinical sinus CT scans of ten female and ten male living patients. The scans were obtained from University College London Hospital (UCLH), having received appropriate ethical approvals. The robustness of each model produced by each observer was assessed through a qualitative assessment and a comparison of sex-determination scores. Each observer scored their models using standard sex-determination methods.<sup>1,2</sup> To further validate this method and the utility of the 3D models, an additional four observers with a background in forensic anthropology were selected to take part in examining and scoring the sex of the original models. To demonstrate the accessibility of this method in a global sense, videos of the models in rotation were provided electronically to the additional four observers. Statistical analysis investigated the degree of observer agreement using Fleiss's kappa and Kendall's coefficient of concordance.

The results demonstrated that all observers successfully produced 20 exportable Stereolithographic (STL) 3D models by following the step-by-step method provided. A qualitative inspection of the models found no major differences to the morphology of the crania models produced by each observer, but some minor discrepancies were seen, for example, in modeling the orbital bones. Statistical analyses showed there was high observer agreement between crania features in the sex-determination scoring and the observer video test scoring.

The findings demonstrated that: (1) users with little to no experience of 3D modeling (or using CT data) could create 3D models using the step-by-step method presented, and (2) consistent and robust crania models were produced, which overall verified the reproducibility of the step-by-step method developed. This method has the potential to provide a standardized visualization procedure, using open source software, that can be implemented reliably in future research and forensic human identification casework.

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### Forensic Anthropology, 3D Modeling, Computed Tomography



## A73 Gerdy's Tubercle Shape and Associated Biomechanical Factors

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**Learning Overview:** After attending this presentation, attendees will better understand the implications of how various shapes of Gerdy's Tubercle (GT) may indicate degenerative changes or habitual activity in an individual, which may contribute to identifying unknown remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing the relevance of GT shape in reference to the biological profile and pathological analysis.

The Iliotibial Tract (IT) is a longitudinal band of fascia that runs from the ilium along the lateral thigh down to the proximal tibia. It has a number of purposes, including stabilizing the knee as well as extending, abducting, and laterally rotating the hip.<sup>1</sup> The IT band inserts distally at GT. Because a number of pathologies due to hyperactivity, such as Osteoarthritis (OA), are associated with the IT band, this research attempted to address several questions.<sup>2</sup> First, is knee OA associated with shape changes of GT? Second, is Body Mass Index (BMI) associated with the shape of GT? Third, does having a physically intensive occupation (e.g., fieldworker) change the shape of GT?

Data was collected on 126 curated tibiae (63 right, 63 left) of both male and female adults from the Texas State donated skeletal collection. GT shapes were separated into four discrete categories: triangular, oval/round, irregular, and unobtrusive.

Data on GT shape, progression of arthritis on the distal femur, patella, and proximal tibia, labor intensity, and BMI were collected for each individual ( $N=63$ ). Each right and left tibia was run separately as many individuals had varying GT shapes and levels of OA per side.

Arthritis was scored using Calce et al.<sup>3</sup> Labor was scored as either non-labor or labor by assessing the individuals' reported jobs or industries. Individuals with unknown work were removed from the dataset.

R-studio was used to analyze the collected data, utilizing a General Estimating Equation (GEE) package. This GEE test was used to analyze any possible correlations between GT shape and side and the various scored factors. A second analysis was run, including side as a factor for possible correlation.

There was no significance when GT was compared with BMI, labor, and femoral and tibial OA. When GT was compared with BMI, labor, and patellar OA, there was significance between GT shape and patellar eburnation ( $\alpha=0.05$ ,  $p=0.0027$ ). However, only one individual had any indication of patellar eburnation, so these results are inaccurately significant due to a small sample size.

A major forensic goal of this research was to assess modern activity patterns and provide a further line of evidence for possible past occupational field or activity level of unidentified individuals. The results, however, provided no clues toward what significance the shape of GT might hold, if any. Future research is needed to diversify the sample and shore up some of the limitations faced with self-reporting.

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### Gerdy's Tubercle, Biological Profile, Degenerative Changes

## A74 Fatal Shotgun Trauma to the Cranium With Discrimination Between Manners of Death

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**Learning Overview:** The objective of this research is to better understand the fracture patterns associated with Shotgun Trauma (SST). This presentation will allow attendees to recognize the differences in expression of trauma between suicide and homicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the importance of examining SST.

Features of cranial Gunshot Wounds (GWS) have been examined for decades, including beveling, fracture patterns, trajectory, wound size to caliber size, and multiple GWS.<sup>1-6</sup> These studies focused on handguns with little-to-no mention of SST, yet approximately 1% of the population dies from SST.<sup>7</sup> Medical Examiners (MEs) often use osteological reports to help inform manner of death, thus anthropologists should be able to recognize the patterns associated with SST.

C.A. Pound Human Identification Laboratory (CAPHIL) cases between 1974 and 2018 were examined for SSTs; 17 cases were found with 15 identified individuals and 2 unidentified. Trauma description was taken from the case report; if the report lacked detail, an analysis was performed using case images. Manner of death was determined by the ME. Fracture characteristics and intrinsic characteristics were also noted.<sup>8</sup> Chi-square tests were done to test for significance.

Sixteen individuals were shot peri-mortem and one postmortem. The postmortem case was not considered further. Of the 15 identified individuals, males ( $n=12$ ) were significantly more represented than females ( $n=3$ ;  $p=.00159$ ). White individuals ( $n=14$ ) were significantly more represented than Blacks ( $n=1$ ;  $p=2.56977E-05$ ). Five of the cases were suicides, two were declared undetermined, and nine were homicides. The weapon was present on scene in all of the suicides and one of the homicides ( $p=.0302$ ). There is no significance between type of pellet and manner of death ( $P=.699969$ ). All cases had comminution, diastatic fractures, and fracture lines crossing suture borders.

Of the suicides, four had trauma documented. All had intraoral or submental entrances; three with an anterior to posterior trajectory and one with an inferior to superior trajectory. Fracturing was too extensive to determine exit. Most of the buttressing elements of the skull were not recovered in three of the cases.<sup>9</sup> Elements such as the occipital were highly comminuted with destruction of the buttressing area.

All homicides had some trauma documented. All but one case had one GSW; the outlier had two GSWs. Three cases had posterior-anterior trajectories; two had left-right; three had right-left; and one was unknown due to trauma. In cases with damage to the frontal bone, nearly half had symmetrical fracturing of the frontal, two had asymmetrical fracturing, and the rest had comminution or were missing the frontal. Half had fracture lines through the buttressing of the temporals. Six had damage to the buttressing of the occipital. All had damaged or missing orbits. One case had fracturing at the buttresses of the zygomatics and one at the mental eminence. Three cases had a LeFort fracture of some type.

Current practice associates distant and/or multiple shots with homicides and close contact shots with potential suicides.<sup>10,11</sup> Three CAPHIL homicides were found with the wad in the cranium, indicating a close distant shot.<sup>8</sup> Multiple SSTs are also considered homicide only; only one CAPHIL case had multiple shots.<sup>10,11</sup> These factors will not help in these cases. Location of SST may be more helpful. Suicides had intraoral/submandibular entrances with an anterior-posterior or inferior-superior trajectory; this is the most common suicide pattern.<sup>13</sup> Homicides had an occipital or parietal entrance with a posterior-anterior or lateral trajectory. There were no anterior entrances, unlike other studies.<sup>14</sup> Another difference between homicides and suicides is the lack of symmetrical fracturing in suicides. The force of the contact shots destroyed the thicker areas of the skull. The face and frontal were often not recovered, possibly due to the fact that these fragments can be ejected from the body from the force of the projectile.<sup>15</sup> Fractures from homicides were more likely to follow cranial buttressing and exhibit fracture symmetry. Examining entrance wound location, trajectory, and fracture patterns is likely to aid in differentiation between suicide and homicide. A larger sample size, including accidental deaths, would be necessary for confirmation. Undetermined deaths will be included in future studies to examine if manner of death may be determined.

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### Gunshot Wounds, Trauma, Shotguns



### A75 Comparing Dental Indicators of Developmental Stress in Unidentified Migrant Remains to Narratives From Living Migrants: A Cross-Disciplinary Approach

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**Learning Overview:** After attending this presentation, attendees will understand how skeletal indicators of childhood stress in unidentified migrants can be used in conjunction with narratives from living migrants to aid the humanitarian efforts at the United States-Mexico border.

**Impact on the Forensic Science Community:** This presentation will impact attendees by discussing the importance of using cultural data collection methods in addition to skeletal data collection methods in humanitarian forensic contexts.

Individuals can biologically embody an adverse sociocultural environment. At a populational level, this can result in physiological health inequalities between groups. This project is an investigation of the concept of embodied inequality in the timing, severity, and duration of Linear Enamel Hypoplasia (LEH) in the dentition of modern presumed migrant individuals found deceased along the Texas-Mexico border and modern White individuals who donated their skeletal remains to the Texas State Donated Skeletal Collection (TXSTDSC). Additionally, narratives collected during interviews with migrants waiting for asylum decisions along the United States-Mexico border are compared to the skeletal data. Using cultural anthropological narratives in conjunction with the biological stress data can be impactful and humanizing, and it can aid in highlighting the ongoing humanitarian forensic crisis at the border.

For analysis of LEH, 30 mandibular canines (15 male, 15 female) were chosen from unidentified migrants curated by Operation Identification (OpID) at Texas State University and 29 mandibular canines (13 male, 16 female) were chosen from donated individuals from the TXSTDSC. These specific teeth are the most likely to show LEH if early life stress is experienced. Teeth were not used from migrants if the individual was positively identified, if DNA sequencing was pending, or if dental analyses had not yet been completed by OpID staff. All canines were viewed under a DinoLite™ for observation and measurement of macroscopic LEH and were subsequently viewed under a scanning electron microscope for identification of microscopic LEH. All LEH was measured and age-at-formation of the stress episode was calculated.

All interviews were conducted at a shelter along the United States-Mexico border, which will remain unnamed to protect the identity of the participants. All permissions were acquired (IRB #17-025). The purpose of the interviews was to better understand the journey that migrants undergo on the way to the United States, and a focus was placed on those who lost a loved one somewhere along the way. Interviews were conducted as conversations, with the participants being allowed control over the flow of the discussion and no pre-established round of questions were asked. Narratives were analyzed for content specifically mentioning children and childhood experiences.

Out of 29 skeletal individuals from the TXSTDSC, 10 (34%) possessed macroscopic LEH and 24 (83%) possessed microscopic LEH. In the migrant individuals, 29 (97%) possessed a macroscopic LEH and 28 (93%) possessed a microscopic LEH. Males (3.13 years) and females (3.34 years) in the TXSTDSC possessed different averages as did the males (3.86 years) and females (3.00 years) curated by OpID. These results can be potentially framed in the biocultural context of child upbringing in Central America versus the United States; however, emphasis is placed on the staggering difference in the stress experiences of migrants and White Americans. Migrants that die while attempting to cross the United States-Mexico border have been experiencing physiological stress since early childhood. This is also reflected in the narratives recounted from living migrants at shelters in Mexico. They discuss the danger and stressful situations their families undergo while living in Central American countries, ranging from threats to murder.

Cross-disciplinary approaches and skeletal stress data are uncommon pairings in forensic anthropology, but they can be quite useful in humanitarian forensic contexts. Narratives can add documented human experiences to quantitative data, which can ultimately yield a more humanitarian or humanizing approach, rather than oversimplifying and inferring stress experiences that cannot be directly observed. Furthermore, while this project does not attempt to positively identify deceased migrants, skeletal stress data and living narratives can aid the ongoing humanitarian crisis at the border in other ways, such as bringing attention to the reasons for migration, the biological costs of migration, and informing policy changes based on quantified and experiential data.

#### Physiological Stress Indicators, Living Narratives, Operation Identification

## A76 Impacts of Biosocial Environment on Developmental Plasticity Among Unidentified Presumed Migrant Skeletal Remains Recovered Along the United States-Mexico Border

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**Learning Overview:** After attending this presentation, attendees will better understand the variation in Vertebral Neural Canal (VNC) asymmetry in samples of presumed migrants from Texas and Arizona, as well as how it relates to potential differences in social disparities relative to region of origin.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the utility of incorporating skeletal indicators of stress into holistic forensic casework. Additionally, this presentation will contribute to an understanding of the skeletal variation in migrant populations that developmental plasticity has the potential to influence.

Stressors experienced during an individual's lifetime (e.g., malnutrition, illness, structural violence, and epigenetic accumulations of trauma) can modify the skeleton.<sup>1</sup> Development of the VNC anteroposterior (AP) and transverse (TR) diameters begins *in-utero* and continues through adolescence, making these structures susceptible to developmental plasticity.<sup>2</sup> While an individual may experience catch-up growth with improved conditions into early adulthood, VNC AP and TR diameters will remain stable after maturation, making them ideal indicators of these earliest stress experiences.<sup>2</sup> VNC asymmetry, the degree of fluctuation in the VNC dimensions from an expected normal, allows for analysis of these stress experiences at the individual and population levels. The continuing humanitarian crisis along the United States-Mexico border demands attention for the expansion of methods employed in the identification process. While studies indicate that unidentified presumed migrants recovered in Texas are increasingly from the "Northern Triangle Countries" and fleeing political instability, gang and drug violence, presumed migrants recovered in Arizona are primarily from Central and Southern Mexico and reflect a displaced rural demographic escaping similar forms of violence.<sup>3-5</sup> The purpose of this research is to improve understanding of the impact the biosocial environment has on developmental plasticity among presumed migrants by examining VNC asymmetry, and to consider how these interactions can inform future forensic anthropological methods employed in this humanitarian crisis.

The skeletal remains assessed in this research are comprised of willed body donations of modern Americans in the Texas State Donated Skeletal Collection (TXSTDSC) ( $n=76$ ), unidentified presumed migrant forensic cases curated at the Forensic Anthropology Center at Texas State under the Operation Identification (OpID) project ( $n=104$ ), and at the Pima County Office of the Medical Examiner (PCOME) ( $n=32$ ). VNC AP and TR diameters of available thoracic and lumbar vertebrae (T1-L5) unobstructed by pathology were recorded according to Watts' method.<sup>2</sup> Demographic information was collected via self-reported questionnaires of willed body donations in the TXSTDSC, and biological profiles were completed by forensic anthropologists at OpID and the PCOME.

These measurements were stratified by sex prior to statistical analysis, and VNC AP and TR diameters were standardized by calculating sample means and individual z-scores. *T*-Tests ( $p<0.05$ ) for vertebrae (along the T1-L5 series) indicate significant difference between the VNC AP and TR diameters of females and males in each sample, yet the vertebrae that differed varied. Analysis of Variance (ANOVA) tests ( $p<0.05$ ) for the VNC AP and TR means between samples found a significant difference between them in both the VNC AP, ( $p=2.1E-13$ ) and VNC TR diameters, ( $p=4.85E-14$ ). Patterns in the spread in variation (z-scores) of the VNC AP and TR dimensions within individuals indicate differences between early childhood and adolescent growth and stress between the samples.

Differences in developmental disruption of the VNC AP and TR diameters among unidentified presumed migrants and modern Americans indicate different early life stress experiences. These processes may inform forensic anthropological methods employed to estimate the biological profile and may indicate an additional avenue to improve population-specific identification methods for the humanitarian crisis along the United States-Mexico border, and beyond. Improved understanding of these skeletal indicators of inequality also hold potential to help the living by providing evidence of the biological repercussions of social disparity and the various forms of violence that migrants seek to escape.

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### Unidentified Migrants, Vertebral Neural Canal, Developmental Plasticity



### A77 The Use of Paid Informants in Post-Conflict Human Rights Contexts

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**Learning Overview:** After attending this presentation, attendees will have a fuller understanding and appreciation of how the use of paid informants in post-conflict human rights contexts can prove, on some occasions, essential in locating clandestine burials.

**Impact on the Forensic Science Community:** Exploring the nexus of: (1) post-conflict forensic investigations; (2) ambiguous loss theory; and (3) critical discussions in the fields of criminal justice and political science, this presentation will impact the forensic science community by introducing the concept of compensating informants to reveal the location of clandestine burials. Enactment of such a proposal would not be without controversy as informants knowledgeable of such burials could have been involved in the crime to some degree, or may even potentially be the perpetrators themselves.

The International Committee of the Red Cross defines Humanitarian Forensic Action as the application of science to the search and identification of missing persons after disaster or conflict. The premise of such work is to mitigate suffering of surviving kin by accounting for the missing, and in the context of post-conflict investigations, is linked to the family's right to *know the fate of the missing* under the Geneva Conventions. Over the past four decades, forensic science has become increasingly engaged in post-conflict investigations, gaining experience in large-scale identification efforts and developing innovative techniques and methods unique to these scenarios. However, one of the hard lessons learned has been that regardless of the efforts and developments put forth by forensic science, search and identification efforts falter when information on clandestine burial locations is exhausted. While scientific methods may find some graves, the prime source of location data continues to be from first and secondary witnesses. Once witness information has been depleted, the search and identification process radically slows, often leaving the investigative organization scrambling to develop additional means of finding graves and struggling to explain the drop in their identification rates to family groups, financial donors, and other supporters.

This presentation proposes compensating informants to reveal the location of clandestine burials in post-conflict investigation contexts. Hold-out informants, those that have not come forward previously with information, are likely individuals that know about the circumstance of disappearance second-hand yet choose not to reveal their information, have been directly involved in the disappearance of the victim to some degree, or may even potentially be the killer. Critics would point out that compensation rewards perpetrators and enablers for their actions, and perhaps it is; however, one can argue that it is wrong *not* to consider incentivizing witnesses in light of the continuing trauma these absences deal to surviving family members and society as a whole.

The loss that family members feel, not knowing if loved ones are alive or not, is a psychological malady known as ambiguous loss, and can prevent survivors from grieving, prolonging their agony. Mass disappearances may also impact the social and economic life of a population for generations, leading to misinformed narratives of the past conflict, mistrust in current governance, and even suspicion about the forensic investigation/ identification process. Thus, ambiguous loss can undermine the very efforts to mitigate the trauma of the conflict that originally motivated the humanitarian forensic investigation.

One can argue that when the point of diminishing returns is reached in an identification process, government/organization leaders should then consider implementing a compensatory policy to entice informant hold-outs to come forth with their information. Further, one can argue that there is precedence of paying informants for their knowledge in the fields of criminal justice and political science. Specifically, police departments regularly pay known criminals to gain information on illegal activity. In international kidnapping/hostage situations, payments to perpetrators for the release of their captives is a common practice, and even unofficial policy for some governments. To enact this compensatory system, a framework must carefully be crafted that includes how best to introduce the policy to the public, protection of informant identities, and a process to validate informant information prior to compensation in order to avoid abuse of the system.

The introduction of a compensatory policy is expected to be controversial; paying potential perpetrators to tell where they buried the body will be viewed as rewarding their actions. Yet, there is solid precedence of rewarding perpetrators for their cooperation in order to allay further harm. To do nothing is to condemn surviving family members to a liminal state of unending trauma, a moral impasse in its own right. Further discussion on this topic will be encouraged.

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#### Paid Informants, Ambiguous Loss Theory, Post-Conflict Investigations





### A78 The Use of an Integrated Multidisciplinary Approach to Resolve Non-Identified Human Skeletal Remains in Cyprus

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**Learning Overview:** After attending this presentation, attendees will be familiar with a novel multidisciplinary approach designed to minimize or resolve cases of unidentified human remains in large-scale humanitarian projects. The model proposes a more interactive and continuous use of investigatory information from the early stages of recovery operations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) introducing attendees to the challenges faced by the Committee on Missing Persons (CMP) in identifying missing persons when the DNA of the recovered remains does not match with any of the Family Reference Samples (FRS); and (2) introducing an integrated multidisciplinary approach for minimizing or resolving cases of unidentified human remains.

In most humanitarian projects, a percentage of the recovered human skeletal materials, after a general search is made in the database of the FRS, remains unidentified. In Cyprus, the list of the missing persons was compiled by the CMP. This was achieved mainly through the cross-checking of the missing persons reported by family members, the army, and by a community member in a position to know all his co-villagers (i.e., the mukhtar of each village). However, soon after the commencement in 2006 of the CMP's Project on the Exhumation, Identification and Return of Remains of Missing Persons, it became evident that not all human remains recovered in the field matched with the FRS related to the initial list of 2,002 missing individuals. By mid-2017, while 886 individuals had been identified, there was a minimum number of 137 individuals that either had no match in the database or did not yield enough scientific evidence to proceed with an identification. Many of these cases remained unresolved for more than a decade in the storage areas of the CMP anthropological laboratory.

This presentation outlines all steps taken to not only reduce this number to one-third in less than two years (current MNI=46), but also to act in advance in order to prevent the occurrence of the no-matches. In this model, the interaction between the investigators and the geneticists was strengthened and included the following: (1) generation of hypotheses on candidates who could be related to a burial site upon recovery of remains, (2) checking the availability of the candidates' FRS even prior to the commencement of the anthropological analyses, (3) revision of all available pedigrees, (4) requests of additional FRS when needed—to accomplish this a project was initiated whereby many skeletal samples were collected from deceased relatives, (5) revision of hypotheses on candidates once the biological profile was completed, and (6) verification of hypotheses once the genetic data was obtained. This resulted in an increased number of the matches during the first round of the genetic database search, thus minimizing the no-match cases and expediting the identifications.

For the generation of the hypotheses, a new approach was introduced whereby missing persons were mapped and linked to events. This led to a better understanding of the context and produced more targeted and limited lists of candidates. In commingled cases, bones were mapped and studied in relation to the identifications/investigative data to produce a better understanding of the expected number of individuals. In addition, in cases in which there was an indication that the remains were not relevant to missing persons (e.g., remains associated with cemeteries or archaeological contexts), the radiocarbon sampling was expedited.

In Cyprus, the human remains recovered by CMP are not always associated with a closed group of missing individuals. By using a multidisciplinary approach, where investigative information plays a central role, it is possible in most cases to either reach an identification or conclusively eliminate the possibility that the remains belong to a reported missing person. Since the genetic identifications have many limitations, such as incidental findings, no available or no informative FRS, partial profiles or very common alleles and so forth, a greater emphasis on the investigatory information can improve the percentage of identifications. Greater awareness on this topic, within the humanitarian forensic world, can reduce the number of unidentified remains.

#### Multidisciplinary Approach, Unidentified Human Remains, Identification of Missing Persons



### A79 What Happens to Unidentified or Unclaimed Human Remains Within the Contexts of Five Asian Medicolegal Systems?

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**Learning Overview:** After attending this presentation, attendees will better appreciate the diversity in the medicolegal systems in Asia, the challenges mass disasters can present in identification, and the manner in which Malaysia, Nepal, Pakistan, the Philippines, and Thailand each manage unidentified human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting how there is not one universal medicolegal system in Asia that will work within every country and how each medicolegal system manages the disposition of unidentified human remains differently.

Asia is a dynamic region that boasts countries with the two largest populations in the world. As such, the medicolegal systems in the region must cope with large numbers of deaths and potential large numbers of death investigations. Due to different histories, and reflecting the cultural diversity in the region with respect to languages, religions, ways of life, and burial customs, different medicolegal systems have been established that respond to the various needs within their geopolitical structures.

This study aims to demonstrate the diversity in the medicolegal systems of Asia by comparing what autopsy means, when autopsy is performed, the mandate for death investigation, and what happens to unidentified human remains, among others, in the contexts of the following five countries: Malaysia, Nepal, Pakistan, the Philippines, and Thailand. In addition to the variables outlined above of language, religion, ways of life, and burial customs, among others, these countries vary in size, population, major religion, and geography—one is landlocked and another is an island nation, for example.

One constraint that appears to be common among all of the countries under study, however, is the lack of storage facilities for long-term, unidentified, or unclaimed bodies. How each country manages their unidentified or unclaimed human remains varies, in part depending upon the dominant religious beliefs of each country. Burial customs in these contexts in Asia range from inhumation burial common among Muslims and Christians to cremation burial common among Hindus and Buddhists. The disposition of unidentified and/or unclaimed human remains in these contexts include marked common graves and mass burials. The timing of when these burials take place also varies within each context. There are a multitude of reasons for why a body is not identified or left unclaimed. One related factor in Asia is that the region is prone to natural disasters in the form of cyclones, tsunamis, floods, and earthquakes. These natural disasters present a risk to the lives of the inhabitants, and further, the risk of not being identified, especially in the case of mass casualties. As an example, there are yet unidentified human remains from the mass disaster that resulted from the 2004 earthquake/tsunami in Indonesia.

What is clear, as the results of this study demonstrate, is that while there is no universal medicolegal system that would work within every country, the various medicolegal systems in Asia presented in this study meet the basic requirements within their own regulations and contexts. Finally, how each country solves the issue of unidentified and/or unclaimed bodies varies vastly, as will be presented.

#### Medicolegal Systems, Asia, Unidentified Human Remains



### A80 Antemortem Data Collection in West Africa as Part of the Effort to Identify Deceased Migrants: A Humanitarian Dilemma

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**Learning Overview:** After attending this presentation, attendees will better understand the technical and ethical challenges involved in the collection of antemortem data for deaths related to migration for humanitarian purposes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the different elements that need to be considered concerning antemortem data collection for migrants from West Africa.

According to the most recent figures from the International Organization for Migration, since 2014 at least 14,000 migrants lost their lives while crossing the Mediterranean Sea.

In January 2017, the International Committee of the Red Cross (ICRC) and the Italian Red Cross signed a Tripartite Agreement with the Office of the Special Commissioner of the Italian Government for Missing Persons to support in the efforts of identification of dead migrants recovered in Italy, with an exclusive humanitarian purpose.

Within this framework, an ICRC project was launched to assist with identification of the victims of one of the largest incidents in the Mediterranean Sea. On April 18, 2015, an estimated 1,000 migrants lost their lives. As part of the project, families of missing migrants potentially affected by this shipwreck are sought in their countries of residence and/or countries of origin, and, if located, are interviewed to aid identification efforts. In Western African countries, where many of the victims are believed to be from, the search for the families remains extremely difficult due to the limited information available.

Moreover, the specific challenges in the collection of the antemortem data from the relatives who reside not only in Africa, but across the globe, has highlighted the need to reframe what is considered useful information for identification. Generally, antemortem data is collected with the underlying assumption that individuals possess specific attributes that will allow them to stand out from a group of individuals. The logic is that physical traits, medical treatment, and lifestyle imprint specific elements to individuals that may, together with other elements, assist in the identification process. This assumes, unfortunately, that most people have access to services that leave a record (e.g., dental records, hospital records, X-rays, etc.). Depending on an individual's and his or her community's circumstances, such services are not always accessible.

Besides the lack of medical and physical information available, differences in culture, language, religion, and family structure references between the interviewer and the interviewee challenge the medicolegal approach to collection and reporting. Not always acknowledged is the collector scientist's expectation that the available data will respond to his/her needs, while the data provided does not easily have an equivalent. For example, a "window" observed between front teeth (emic) as described by a relative, could be described as a diastema or a missing tooth in the dental or postmortem examination (etic). The comparative data that is required for scientific identification may exist, but not in a format familiar to the scientist.

At the same time, genetic identification in the migration transnational context has its own challenges in terms of how data is shared and stored as well as how collection is planned. For example, in the collection of genealogical data to support collection of Biological Reference Samples (BRS) from surviving family members, several assumed categories such as cousin, brother, or sister may not correspond to biological relationships needed for genetic testing. In addition, common to West African culture, any child adopted into the family automatically becomes a son or a daughter and will be referred to as such. Even descriptions of personal effects (specifically clothing) may be unreliable as these are easily changed after departure up to embarkation.

Experience has shown the need for an innovative, pragmatic humanitarian approach to antemortem data collection in the migration context. Usually antemortem data includes information on missing persons, notably personal, physical, medical, and dental information, as well as information on circumstances related to the disappearance. A reflection is needed, though, on methods to better exploit circumstantial and contextual information (e.g., the trip itself, fellow travelers) coupled with the usually scarce individual biological data.

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#### Migration, Antemortem, West Africa



### **A81 Climate, Conflict, and Migration—Applications of Forensic Science to Address Humanitarian Issues in a Growingly Complex Global Environment**

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**Learning Overview:** After attending this presentation, attendees will better understand the broader connection between the growing challenges brought about by climate change, and how it is impacting armed conflict and migration, and the humanitarian challenges that stem from these issues. Attendees will learn about the importance of understanding cultural and religious diversity, how this impacts on a transnational problematic, and how forensic sciences can transcend these issues and help address these growing humanitarian challenges.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the importance of introducing thinking surrounding cultural and religious issues on how they work and look to address the growing challenges in identifying human remains through understanding the root causes of this problem and will help forensic practitioners develop better models for approaching this complex issue.

When we think of crossing borders, we often see this as simply a physical barrier; for example, passing from one state into another. The movement of people globally, whatever the reason may be, has created much greater borders than simply physical ones. We live in a growingly globalized world, and the impact this has when we look at the problematics of migration, conflict, and climate only go to reflect this. Our connectivity plays a great role in this, breaking down what were traditional borders and allowing us to live in a very interconnected ecosystem. This same connectivity allows us as forensic scientists to attempt to address what often seem insurmountable challenges, especially when they relate to trying to determine the origin and identity of someone who is found dead along the large number of human paths that transverse our planet.

Pushed out of their homelands by the growing impact of climate change, which often fuels conflict over already-scarce resources and land, the numbers of persons perishing throws down some of the greatest challenges facing us as forensic scientists as we grapple with the task of trying to find a way to ensure the handling and disposition of the dead, in line with the legal, cultural, and religious needs of someone we may know nothing about, and the task of attempting to give this person back their name.

With communities now living transregionally, connected by a growingly integrated communications network, the idea of country of origin and destination for migrants tends to blur. The efforts of forensic science can bring together an interconnected network of family members to try and piece together the many fragments of information in the efforts to identify a missing relative. However, this can leave forensic practitioners and all those involved in the search process trying to navigate the complex patchwork of culture and society.

A growing recognition and engagement of the forensic community in humanitarian and human rights issues is providing hope. Advances in areas such as forensic genetics, the use of stable isotopes, and complex network analysis of population movement all are playing an important role. However, forensic science alone will not address these challenges. Major legal considerations must be understood, for example, in terms of cross-border transfer of data. With such divergent systems in place across the globe, this only further underlines the need for the harmonization of systems and procedures between and within states to allow for better transfer of necessary information in a format that can be used both practically and legally by all concerned.

Understanding climate change and how it will impact conflict through the battle for scarce resources, forced displacement from flooded areas, or cities no longer able to provide sufficient water for inhabitants will be essential as we look to the real impact this will have on the death of civilians in the years to come. Understanding how this will impact communities will be essential as we try to understand what role forensic science will inevitably play in trying to recover and, where required, identify the victims of these realities of the future.

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#### **Climate Change, Armed Conflict, Humanitarian Forensics**



### A82 Crossing Borders: Conflict Evolution and Climate Change—The Humanitarian Consequences on Human Migration

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**Learning Overview:** After attending this presentation, attendees will understand how displacement and its temporal dimensions affect processes of recovery and identification of persons missing as a result of violent conflict, specifically in relation to delayed responses and prolonged uncertainty.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the temporal elements and obstacles inherent in forensic efforts to recover and identify missing/unknown persons and how those, in turn, affect surviving kin and their perception (and reception) of the forensic processes. In doing so, it highlights the asymmetrical relationship between those controlling and implementing the forensic response and those awaiting its results.

From the pressure to locate remains, collect and analyze evidence, and deliver definitive results, humanitarian responses to incidents of mass fatality are often cast—understandably so—in terms of the urgency of time. A race against the proverbial clock, forensic efforts grapple with time-sensitive conditions as varied as environmental exposure and degradation and the fading memories of potential witnesses to clandestine burial. Yet time is a complicated metric when applied to the identification of victims of mass fatalities, particularly when it comes to the social consequences of violent conflict and mass displacement.<sup>1</sup> Grounded in the premise that “time and space are integral to each other,” this presentation focuses on these consequences to illustrate how such displacement introduces obstacles and often delays in processes of recovery and postmortem identification.<sup>2</sup> It also invites a more nuanced consideration of how prolonged uncertainty shapes the way families perceive and evaluate the efficacy of forensic responses. Finally, time as an analytic sheds light on the interplay between humanitarian and political aims underwriting forensic efforts to reclaim and return the remains of victims of violent conflict.

Time unfolds unevenly, in war and its aftermath. The seconds it takes to destroy a single life or the hours involved in the illicit disposal of bodies may require years, even decades, of investigation—archaeological, anthropological, and genetic—to reassociate an individual identity with a set of unnamed remains. All the while, relatives of the missing wait: for news of life, of death, and eventually of recovery and return. The disparity between these temporal dimensions is compounded when conflict scatters not only the bodies of the missing, but also those who survived them. Two examples document this asymmetry and its import. The first relates to forensic efforts to identify the 31,000 missing persons from Bosnia and Herzegovina, specifically the more than 8,000 Bosnian Muslim (Bosniak) men and boys missing as a result of the Srebrenica genocide, where mass displacement of surviving relatives complicated both the DNA-led forensic response and the community’s ability to mourn for and commemorate its victims.<sup>3</sup> In the Srebrenica case, the tension between the urgency of immediate postwar humanitarian action and the longer-term memory politics of national belonging emerge most sharply at the annual commemoration and mass burial of identified victims in the communal cemetery of the Srebrenica-Potočari Memorial.

The second example addresses the United States military’s decades-long efforts to account for its service members Missing In Action (MIA) and presumed dead from the Vietnam War. Akin to the recent initiative to identify Argentina’s unknown soldiers buried on the Malvinas Islands, although set to the political calendar of shifting administrations and geopolitical relations, the American MIA accounting mission in Southeast Asia is explicitly termed a “humanitarian” program. Gradual diplomatic rapprochement has indeed opened doors for archaeological recoveries and eventual identifications. But time has also adversely affected the mission’s results. Landscapes are altered; remains are degrading; witnesses are dying, and memories fading. Beyond these physical and social forms of decay, time also troubles surviving kin. Some go to their own graves without burying their loved ones. Outsized expectations further fray trust in expedient results and prolonged uncertainty exacts tolls that not even the most certain of forensic evidence can assuage.<sup>4</sup>

In examining the temporal dimensions of conflict, displacement, and delayed care for the dead through the examples of postwar Bosnia and Herzegovina and contemporary United States, this presentation demonstrates the sociopolitical complexities of humanitarian forensic intervention when time is continually of the essence, but results come decades later.

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#### Displacement, Humanitarianism, Time



### **A83 Crossing Borders and Conflict: The Complex Circumstances of Those Who Disappear in Armed Conflict and the Simple Duty of Care to Bring Them Home**

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**Learning Overview:** After attending this presentation, attendees will better understand how cases from different past and contemporary conflicts can address questions of how, why, and when to seek out the remains of the “military” dead and how they ought to be treated. Attendees may also understand how modern borders are reconciled to ensure that different forms of care for the dead (identification, commemoration, repatriation), without making judgements about their motives for, or agency in, crossing borders is both respectful and sustainable.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reexamining the treatment of military dead through the lens of migration and uses several cases: a woman who longs to give dignified burial to her family that was killed in conflict in their homeland; a mother who clandestinely recovered the remains of her soldier son buried abroad; and the ramifications of nationality and place of death on how governments and families commemorate their dead. This report will present solutions to ethical and sustainable management of those dead due to armed conflict.

While the scale of recent migration and subsequent deaths in places such as the Mediterranean Sea and the United States-Mexico border has been shocking, the death of large numbers of foreign nationals en route to another place or while in another country is nothing new. We do not always see armed actors as migrants, but indeed, the challenges of managing their deaths overseas, the circumstances of their deaths, and the sociopolitical policies that impact whether they are sought and repatriated present some similarities as well as stark contrasts to those communities currently faced with the care of the marked and unmarked graves of the migrant dead. Even many years after the end of a conflict, the bodies of armed actors are discovered by happenstance or even actively sought, and how these remains are managed is a story of nationality and place of remains, a story of where “home” and family are for those remains.

This presentation will examine the distinct circumstances that illustrate the fate of those who take up arms, die, and disappear in a place that is not their home. Some choose to take up arms, later dying in countries that are not their own, while others are conscripted to military service and forced to fight, die, and disappear in a foreign land. Others still are forced to flee one type of violence and, being in an extremely vulnerable position, are recruited into armed conflict in their new “home.” This creates a stark contrast—perhaps no less tragic—with those who die while fleeing their home to escape violence.

Often, at the time of death and burial in armed conflict contexts, identification techniques of the dead and the ability to trace surviving families is limited by resources and the exigencies of war. Sometime afterward; however, there are considerable opportunities to seek out the missing dead, identify them, and return their bodies to their communities and families. This presentation will use concrete cases to illustrate the existing legal, technical, and ethical challenges faced by governments, scientists, communities, and most importantly, aging families in the conflicting borders of managing those who bear arms, die, and disappear in armed conflicts.

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#### **Borders, Armed Conflict, Repatriation**



## A84 The Role of Civil Society and the State in Transnational Identifications at the United States-Mexico Border

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**Learning Overview:** After attending this presentation, attendees will learn the discordance between state-mandated legal approaches to identification and humanitarian efforts when dealing with transnational identification efforts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by indicating the need for civil society-based approaches to aid in identification efforts across transnational borders.

Forensic anthropologists often work with unidentified human remains with the goal of building a biological profile to facilitate identification and repatriation. While it was once difficult to share missing person's information across state lines, the past 20 years have seen incredible strides with the creation of the National Missing and Unidentified Persons System (NamUs) and the Combined DNA Index Systems (CODIS). NamUs and CODIS both allow data sharing across jurisdictional lines within the United States and work to associate missing persons with unidentified persons, resulting in fewer unsolved cold cases. Although these systems exist through federal funding, there are no state or federal resources that allow for transnational sharing of missing persons information or genetic data.

The continually occurring mass disaster at the United States-Mexico border, with more than 6,000 reported migrant deaths since 2000, remains challenging to forensic practitioners, families of missing migrants, and all agencies working toward positive identification on both sides of the border.<sup>1</sup> Due to the lack of federal- and state-mandated resources for transnational collaboration regarding unidentified human remains found in the United States, civil society plays a large role in facilitating identifications by acting on behalf of families of the missing as well as working directly with forensic practitioners across borders. The purpose of this presentation is to highlight, through data and case studies, the role civil society has played in the identification of missing migrants along the United States-Mexico border with specific reference to Texas.

Data were collected from the Forensic Anthropology Center at Texas State (FACTS) where 287 migrant remains are undergoing anthropological analysis pending identification through coordinated efforts with governmental agencies and civil society. FACTS, through Operation Identification, works with government agencies and civil society organizations, including the Argentine Forensic Anthropology Team (EAAF), the South Texas Human Rights Center, and the Colibrí Center for Human Rights, to facilitate identifications. As required by state law, FACTS submits DNA samples from unidentified human remains for genetic profiling and upload to CODIS. Additionally, FACTS collaborates with the EAAF and Colibrí to compare unidentified genetic profiles with family DNA data that is not in CODIS. In total, FACTS has facilitated 14 genetic associations through the submission of 55 DNA profiles to the EAAF and 19 genetic associations through the submission of 188 DNA samples to CODIS.

Although FACTS follows state laws regarding unidentified human remains, without additional collaboration with civil society organizations, only 16 out of 33 identifications would have been possible. This collaboration has also led to the discovery of burials representing the long-term dead in need of exhumation and identification efforts. Additionally, civil society has been able to raise awareness of the lack of transnational data sharing to policy makers who have the power to provide much-needed change. The identification comparisons will be discussed within a larger framework using specific case study examples and highlighting specific roles that civil society has played in shaping policy for unidentified human remains.

While working within the United States, individual states have the responsibility to facilitate identifications using either local or federal systems. Although our current federal systems facilitate sharing of information across jurisdictional lines, it is not currently possible to share such information on a transnational level without civil society collaboration.

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### Transnational Identification, Civil Society, Migrant



## A85 Empathy and the Identification of Missing Migrants

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**Learning Overview:** After attending this presentation, attendees will understand the importance of applying an empathetic approach in forensic casework, particularly when attempting to identify missing migrants.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of empathy as a tool for emotional regulation and as an investigative mechanism for identification.

As previous research has demonstrated, assertions of pure objectivity in forensic anthropology are problematic. Forensic practitioners are shaped by their environments, which results in degrees of implicit bias. The lack of acknowledgement of subjectivity is problematic as it can unknowingly guide forensic analyses and interpretations. One aspect of subjectivity that is largely neglected in the forensic sciences is that of emotions, particularly when working with unidentified remains. The prevailing paradigm mandates that forensic practitioners be guided solely by “logic,” but neglects to acknowledge that practitioners are humans who feel emotions, such as anger, fear, disdain, and grief. Strategies to curtail emotion often revolve around desensitization tactics, construction of emotional barriers, and/or the objectification of deceased persons (e.g., not analyzing a human but a bone). The dismissal of one’s emotion may provide the practitioner with a feeling of distance and control, but its efficacy as a mode of objectivity is questionable. It is argued that engaging empathetic processes is one way in which forensic scientists can become more aware of their internal emotional states, better informed of the populations they serve, and more cognizant of novel investigative mechanisms to facilitate identification.

As demonstrated by the Latin American experience beginning in 1983, empathy can lead to *holistic* approaches in forensic science. Empathy refers to a process by which one comes to understand the perspectives of people who are different. Empathy represents a first step in navigating one’s own emotions in order to pursue “mitigated objectivity.” The forensic science community has been slow to understand, embrace, and apply empathetic approaches given the perceived contradiction to scientific “objectivity.” To those unfamiliar with empathy, it is often conflated with sympathy or pity, and viewed as the shedding of detached analyses in favor of unhinged emotion, which is fallacious. In actuality, empathy can contribute to knowledge production and problem-solving through emotional regulation and recognition/interpretation of key features pertinent to the identification process.

This presentation outlines key components of empathy that forensic practitioners should consider in their casework. Components include: acknowledging one’s own involuntary affective responses; recognizing a separation between oneself and others; exposing oneself to the experiences/perspectives of others; cognitively assessing differences/similarities between oneself and others; and regulating one’s emotions. Social science research has demonstrated that effectively engaging empathy provides a mechanism to explore implicit biases and assumptions, and a means to successfully manage one’s own emotional responses (a.k.a. emotional regulation). This process can also provide an overall strategy in the attempt to identify the remains of vulnerable or at-risk groups, such as migrants.

Those who engage in irregular migration represent highly stigmatized and marginalized groups, not only in countries of destination, but also in countries of origin and transit. Migrants are also particularly vulnerable for going missing at various points along their journey and for remaining unidentified even when medicolegal structures are well developed. Drawing on case studies, this presentation demonstrates how an overall lack of empathy toward migrants affects the ability to identify their remains and return them to families. If personal identification is the ultimate objective of analyses, forensic scientists can contribute to this goal and reduce the further devaluing of migrants by becoming familiar with their perspectives in life, by recognizing their group affiliation in death, and by devising effective strategies for their identification (e.g., consultation with community partners).

Applying biocultural profiles for decedents, especially for unknown persons, represents a first step in empathetic engagement. The biocultural approach consists of analyzing material culture, scene context, and biological indicators in a thoughtful manner to develop hypotheses of group affiliation of decedents (e.g., migrants). While employing empathy may elevate the pursuit of justice in clarifying the fate of the missing, it does not decrease objectivity. Rather, the recognition of deceased persons as once-living individuals who belonged to particular communities permits forensic practitioners to better account for mortality trends in certain communities and to investigate appropriate routes for identification.

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### Empathy, Identification, Missing Migrants





## A86 Assembling a Forensics of Structural Violence

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**Learning Overview:** After attending this presentation, attendees will recognize ways that forensic science can contribute to making structural violence visible and responding to it, along with some of the challenges that are likely to accompany those efforts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying specific areas where the ethics and the organizational practice of forensic investigation must adapt to address structural violence.

Beginning with efforts to locate clandestine graves and identify victims of enforced disappearance in Argentina in the 1980s, scientific experts and families of the missing have created a new global paradigm for forensic investigation that is sometimes called “forensic humanitarian action,” a blend of 19<sup>th</sup>- and 20<sup>th</sup>-century ideas of humanitarianism and human rights.<sup>1,2</sup> This presentation argues for complimenting the field’s focus on war crimes, genocides, and “natural” disasters with an integrated, 21<sup>st</sup>-century understanding of structural violence.

Structural violence refers to the differences in people’s well-being or “life chances” that result from “the unequal distribution of power and resources.”<sup>3,4</sup> It is violence that is “built into the structure of society itself.”<sup>5</sup> Critics argue that the human rights movement has long focused too narrowly on direct violence while turning away from the everyday structural violence experienced by the global poor.<sup>6-8</sup>

For forensic scientists, responding to this critique would require moving beyond the traditional definition of “wrongful death”—a claim against a specific perpetrator—to a focus on *preventable* deaths and the living communities impacted by them.<sup>9</sup> Preventable deaths result from disease, poor labor conditions, mass migration, environmental destruction, and the global failure to deter climate change and the unequal distribution of its effects. In many cases, these deaths produce bodies that go missing or wind up in marginalized burial places, their stories never told.

Experts working to identify migrants who die in the United States-Mexico borderlands have called for a forensics of structural violence, and explored its ramifications for the role of individual experts.<sup>9,10</sup> This presentation builds on their work through an interdisciplinary approach combining social anthropology, political theory, and historical perspectives. It further assembles a picture of the forensics of structural violence, as well as ethical and political dilemmas that accompany its necessary development.

A focus on structural violence includes asking what care for the dead—and the chronic disruption of this care—means for the health and well-being of entire communities. It also means questioning what types of historical violence merit forensic investigation: wars and massacres only, or also the forgotten graves produced through generations of exclusion and structural violence?

The forensics of structural violence spark difficult conversations about neutrality and objectivity, and who is present and heard in the spaces where forensic humanitarian organizations chart their directions. These organizations will also need new fundraising solutions to emerge from a humanitarian tradition where “savage inequalities,” though claiming lives every day and disrupting the care of the dead, do not command the same attention as direct violence.<sup>11</sup>

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### Human Rights, Structural Violence, Migration



## A87 Beyond Identification: Structural Vulnerability and the Investigation of Migrant Deaths

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**Learning Overview:** After attending this presentation, attendees will learn about structural vulnerabilities affecting undocumented migrants crossing the United States-Mexico border and living in the United States, and how these vulnerabilities impact migrant deaths and death investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) highlighting the unique perspective and skills of forensic anthropologists that can bring awareness to this continuing humanitarian crisis; and (2) initiating a discussion of the ethical responsibilities of forensic anthropologists working with vulnerable migrant populations.

Nationally, people of color, migrants, transients, and homeless persons are overrepresented in forensic casework, especially in cases of long-term unidentified decedents.<sup>1</sup> Both at the southern border and nationally, undocumented migrants represent a substantial number of these long-term unidentified decedents. However, people of color, and especially undocumented migrants, are immensely underrepresented in local missing persons reports and cases uploaded to national databases. This disparity will intensify as migrants continue to die in large numbers at our southern border and disproportionately within our local communities. As an integral part of the death investigation process, forensic anthropologists are challenged with the identification and repatriation of undocumented individuals, and as a result, we as a discipline are directly and intimately witnessing this humanitarian crisis.

With widely applicable skills in academic, medical examiner/coroner, government, and human rights contexts, forensic anthropologists are in a position that few other forensic practitioners share. Whether attempting to identify unknown remains, contributing skeletal information to the cause and manner of death, or conducting research that will assist in these analyses, we inevitably come into contact with the physical bodies of vulnerable individuals. Many forensic anthropologists also come into contact with distressed and vulnerable families as we communicate with loved ones for the purposes of identification and repatriation. Regardless of the jurisdiction or context in which we work, or the type of analyses in which we participate, as forensic anthropologists we should be aware of the structural vulnerabilities affecting the physical bodies we examine and the families we serve.

This presentation will review structural vulnerabilities in undocumented migrant groups using the three forensic temporal perspectives of trauma as an analytical framework: (1) Antemortem: biological indicators of poverty and physical violence experienced in life and incorporated into the body; (2) Peri-mortem: indicators of structural vulnerability that correlate with the recovery location of deceased migrants and the cause and manner of their deaths; and (3) Postmortem: indicators of structural vulnerability in the unequal treatment of migrant bodies, including incomplete forensic investigation of their deaths, and the burdensome position of family members with a loved one who died while undocumented. These issues will be discussed as they pertain to ethical considerations in research and when handling the remains of vulnerable individuals.

Incorporating knowledge of structural vulnerabilities in undocumented migrants into a forensic context is meaningful for a number of reasons. First, recognizing biological indicators of poverty and physical trauma as additional scientific data can substantially contribute to identification efforts. Additionally, comprehending the stressors affecting families of missing and deceased undocumented migrants may help investigators develop strategies to identify their loved ones and make notification. Undocumented individuals and their families are essentially living “invisibly” in our society and forensic practitioners need to account for this in any efforts to make an identification and when communicating with family members. Some investigative strategies and resources to work through the identification of a probable undocumented migrant will also be presented.

Finally, and most important, there is an ethical responsibility that we must consider in our multidisciplinary role as forensic scientists and anthropologists. Practicing forensic science is to serve the deceased, their families, and ultimately the greater community. As forensic anthropologists, we are deeply involved in various stages of the death investigation process and are in a unique position to share our scientific knowledge and experiences of how this crisis is affecting the greater migrant community. Our service, our research, and our outreach can not only contribute directly to the death investigation process, but also to an awareness of the larger humanitarian crisis that we are witnessing.

The views and opinions expressed in this abstract are the author’s own and do not reflect the opinions of the New York City Office of Chief Medical Examiner or the City of New York.

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### Structural Vulnerability, Undocumented Migrants, Death Investigation



## A88 Families of the Missing: Forensic Obligations to Families During the Identification Process

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**Learning Overview:** After attending this presentation, attendees will leave with a useful framework for ethically engaging with families of missing migrants during the forensic identification process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing ethical guidelines tailored toward protecting and respecting families of missing migrants. Adhering to such guidelines will improve rates of identification as well as the social outcomes of the identification process.

Modern international borders pose both unique and familiar problems to the field of forensic anthropology. From the condition of remains upon discovery to bureaucratic and jurisdictional ambiguities, forensic scientists face deep challenges in their work to identify the dead. However, these difficulties pale in comparison to those faced by families of missing migrants. They often live in a different country from where their loved one disappeared, do not speak the same language as forensic investigators, and face safety and security threats due to their status as migrants themselves or as residents of politically unstable countries. All of this is on top of the trauma that any family of a missing person endures. These sociopolitical issues impact the forensic process and should inform the ethical approach of forensic practitioners involved in any stage of the examination and identification process.

The context of extreme vulnerability for most international migrants means that special care must be taken to interact with families in a way that is ethical. There are three stages in the forensic investigation process, during which time special ethical considerations should be considered by forensic practitioners: (1) discovery and reporting, (2) examination and identification, and (3) release of remains. The following guidelines are based on extensive experience working closely with families of Latin American migrants who have disappeared in Mexico or the United States. These guidelines are also informed by the International Committee of the Red Cross (ICRC) Handbook, “Accompanying the Families of Missing Persons.”<sup>1</sup>

During discovery and reporting, forensic practitioners should understand that one of the primary challenges families face in searching for a missing loved one is reporting. Families of missing migrants are often unable to report to United States law enforcement due to local jurisdictional boundaries, fear, language, or a combination of these. Forensic practitioners have a variety of options to support the family reporting process, including working closely with foreign consulates and Non-Governmental Organizations (NGOs), supporting the family in reporting to law enforcement, or taking a forensically relevant missing person report one’s self.

In addition to following established ethical guidelines and laboratory Standard Operating Procedures (SOPs), forensic practitioners can be mindful of practices that can ethically protect the needs of families of missing migrants during the process of examination and identification. As so many migrants are discovered without identification media, or with false documents, the first ethical consideration is to treat remains as unidentified until a strong correlation is made with a missing person’s report submitted by a family. Family must be involved directly or through a third party such as consulate or NGO for an identification to be valid. Other ethical considerations have to do with the language used and imagery collected during examination. Dehumanizing language and hate crimes against Hispanics and Latinos are increasing nationally.<sup>2</sup> Forensic practitioners should consider the impact of the language they use, not only on the family but also on public discourse. Similarly, photographs should be evaluated for their potentially harmful impact on next of kin or public discourse before being shared with the press, on social media, or in public presentations.

Finally, special ethical considerations should be made during the release of remains as either identified or unidentified. In both cases, it is recommended that any personal effects or property are kept separate from the remains and accessible by next of kin. Also in both cases, cremation without consent from family should be avoided at all costs. In the case of identified remains, the notification process is one of the most ethically complicated areas of work, challenged by the fact that most families of missing migrants live very far from the forensic investigation facility. This presentation will include best practices for the ethical notification of families of missing migrants, including examples of translated identification reports that can be made available to next of kin.

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### Ethics, Families, Migrants



### A89 Revisiting the Concepts of “Race” and “Ancestry” Regarding Missing Migrants in the United States

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**Learning Overview:** After attending this presentation, attendees will understand the complicated history that anthropology has with the concepts of “race” and “ancestry” and how those knotty concepts continue to permeate the forensic identification process at the United States-Mexico border. Attendees will also better understand both the utility and dangers inherent in applying ancestral assessments specifically to missing migrant populations in the United States.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by generating a necessary discussion about the applicability of ancestral assessments to the forensic identification process and what it means regarding an individual’s identity. This presentation will contribute to the discourse by specifically investigating the utility of ancestry assessments on missing migrants.

The use of “ancestry” in the biological profile has its roots in the foundations of physical anthropology. These roots stem from the beliefs that there were true biological differences across various human populations and that certain groups of people were markedly different. The originators of these ideas believed that humans could be grouped into distinct “races,” or even that certain groups should be categorized as separate “species.” This vein of thought reduces individuals into racial typologies and does not adequately represent the true range of human variation. Contemporary anthropologists agree that “race is a social construct,” however this phrase belies the reality that the foundations of much of the current use of “ancestry” in the biological profile can be traced back to a problematic history. Modifying the terminology from “race” to “ancestry” is meaningless with the persistence of remaining underlying issues.

Currently, ancestral assessments utilize a range of different approaches including metric, non-metric, and macromorphoscopic methods. These techniques require an understanding of the range of biological variation and how it is expressed across populations, allowing anthropologists to rely on their experience to ascribe an ancestry estimation to an unidentified individual. In a forensic context, ancestry has historically been divided into discrete categories with varying terminology, such as “European American,” “African American,” etc. This type of categorization does not necessarily represent a decedent’s identity, particularly in instances in which an individual’s ancestry reflects a complex colonial history. Furthermore, there has been little, if any, reflection by forensic anthropologists based in the United States on how “ancestry” estimates are utilized in medicolegal investigations after the anthropological analysis has concluded. Research by scholars outside of anthropology have noted bias in media coverage related to missing persons cases and an unequal emphasis on resources allocated to certain classes of missing people. For example, “Missing White Woman Syndrome” has been described by scholars in criminology and empirical analyses of the Federal Bureau of Investigation (FBI) data have confirmed the reality of this phenomenon.<sup>1</sup>

By extension, the issues with ancestry and identity become magnified when dealing with migrant populations. Consider the example of migrants who perish along the United States-Mexico border and on-going efforts by forensic anthropologists working toward identification. An ancestry categorization as “Hispanic” or “admixed” does not help discern the actual geographic origins of an individual and does little to assist local medicolegal investigators. Treating such a diverse group as a monolith may dramatically reduce the efficacy of any ancestral assessments and has the potential to hinder the identification process. Furthermore, the marginalized and dehumanized status of migrant populations has also played a role in hindering the identification process.<sup>2</sup> Turning to research outside forensic anthropology, scholars in media studies have documented unequal treatment of Latina women who have been victimized by crime or who represent missing persons cases. Ultimately, forensic anthropologists working on migrant identification may need to acknowledge the structural barriers which characterize the medicolegal system in the United States and the reality that not all decedent deaths, and especially those of migrants, receive equal treatment at the conclusion of anthropological analyses.

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#### Migrant, Ancestry, Identity



### A90 Policy, Ethics, and the Use of DNA in Migrant Families

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**Learning Overview:** After attending this presentation, attendees will understand the ethical implications of the policies informing the use of DNA to: (1) screen for human trafficking; (2) reunify migrant children separated from their families; and (3) verify claimed biological relationships in migration.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees on the utility and limitations of DNA testing of families in the context of migration at the United States-Mexico border.

United States border security protocols are turning to DNA as a biometric for processing refugee claims and to screen for human trafficking. Most migrants fleeing their home countries travel without proof of identity, so genetic information is useful for establishing identity and verifying biological relationships. The United States has had the authority to collect DNA from immigrant detainees for the Combined DNA Index System (CODIS) since 2008, primarily to detect immigrants who commit crimes in the United States. Recently, the United States has expanded the uses of biological relationship testing to verify relationship claims in immigration. In 2018, the “zero-tolerance” policy to separate migrant children from the adults accompanying them led to subsequent offers of genetic testing for reunification and use of DNA testing for verifying biological relatives. These discussions sparked controversy in news and social media. Public debate continued into 2019, fueled by a rapid DNA program at the border to perform kinship analysis to verify migrant family claims.

As part of this research into how genomics is used in immigration contexts, this study evaluated how the use of DNA in a politically charged circumstance was covered by news and social media. Systematic searches of newspaper databases and Twitter® were conducted for coverage of DNA testing and migrants for the 2-month period of June and July 2018. For comparison, these search parameters were repeated in May 2019 to capture articles and tweets about the rapid DNA program. This study identified trends, including common topics and miscommunications, as well as significant gaps in discussion of DNA testing and topics covering the legal contexts and ethical implications of using DNA tests as a measure of family relationships. In both news articles and tweets, it was found that a politically conservative or pro-zero-tolerance policy slant strongly corresponded to a pro-DNA testing stance, while a liberal or anti-zero-tolerance policy slant corresponded diversely to pro-, anti-, and neutral stances on DNA testing.

Public discussion surrounding the use of DNA for documenting families demonstrates both the complexity of this application as well as key communication issues in the public sphere, including: (1) lack of transparency on the use of DNA tests (causing speculation); (2) ineffective inclusion of experts in public conversations (causing misinformation to spread); and (3) ill-preparedness of the scientific community to react to public confusion and outcry (causing scientific misunderstandings to go uncorrected). The expanded uses of genetic information beyond crime investigations could result in stigmatization of individuals or entire populations, if applied broadly. Moreover, the geneticization of families undermines the social constructs that underlie human relations and self-identity and could lead to discrimination of non-traditional families or revelations of unintentional family secrets that could endanger individuals.

#### DNA Testing, Immigration, Ethical Implications



### A91 Counting the Uncountable: Certification of Deaths in the Borderlands

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**Learning Overview:** After attending this presentation, attendees will better understand the Centers for Disease Control and Prevention (CDC) guidelines for certifying disaster-related deaths and how the application of these guidelines allows for a better accounting of deaths occurring during irregular migration.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how increased interjurisdictional consistency in investigating and certifying deaths can better inform public health and public safety initiatives related to irregular migration.

During the process of death investigation, Medical Examiners/Coroners (ME/C) routinely collect and report information that may be used for public health surveillance. Death certificate data is used to develop public health and public safety initiatives and inform citizens about patterns and trends in population health. Death certificate data from mass fatality events is used to assess the scope of an event, understand mortality patterns, and evaluate short- and long-term public health impacts. Inconsistencies in certifying a death as disaster-related make it difficult to generate reliable and accurate mortality statistics and to identify the most frequent causes of death associated with disaster events.<sup>1</sup>

Worldwide, it is estimated that, from 2000 to 2019, more than 60,000 people have died during the process of irregular migration. An estimated 6,000 to 7,000 of these migrants have died in the United States as a result of crossing the United States -Mexico border, a death toll similar to Hurricanes Maria and Katrina combined.<sup>2</sup> While alarming, these numbers are minimum estimates only; due to the nature of irregular migration, many of these deaths occur in clandestine fashion and an unknown number of deaths are unreported or undiscovered each year. Moreover, these numbers are a tally only of deaths and disappearances during the process of crossing the physical border and do not reflect persons dying as a result of irregular migration but not in the act of crossing the border (for example, those dying in immigration detention facilities or in hospitals while in the custody of federal law enforcement).

Different government entities and non-governmental organizations may have different methods for counting migrant deaths. There is some evidence that federal data may vary widely from local- and state-level data, hampering policymakers and researchers. Even at the state or local level, ME/C inconsistencies in investigating, certifying, and tracking these deaths make it difficult to accurately count them in a given jurisdiction, much less place deaths occurring at a certain locality within the larger context of irregular migration. Because many of these deaths will fall within the statutory authority of medical examiners and coroners, ME/C data, if comprehensive, can provide the best information about migrant deaths in a given time or place. One way to improve data collection is to standardize the certification of these deaths.

The CDC has published guidelines for the certification of deaths directly and indirectly related to a disaster, whether that disaster is natural, human-induced, or chemical/radiological. This presentation will provide an overview of these guidelines and discuss how they may be adapted to the certification of deaths related to irregular migration. Although the true number of migrant deaths may be impossible to ascertain, better characterization of the known deaths can diminish the magnitude of underreporting of these deaths and allow for improved understanding of the scope of this public health crisis.

**Reference(s):**

1. *A Reference Guide for Certification of Deaths in the Event of a Natural, Human-induced, or Chemical/Radiological Disaster.* (<https://www.cdc.gov/nchs/data/nvss/vsrg/vsrg01.pdf>).
2. The International Organization of Migration (IOM)'s Missing Migrants Project Migration Data Portal (<https://www.migrationdataportal.com/themes/migrant-deaths-and-disappearances>).

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**Disaster Mortality, Irregular Migration, Migrant Death**



## A92 The Detection and Characterization of Human Decomposition Products by Infrared Spectroscopy

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**Learning Overview:** After attending this presentation, attendees will understand: (1) how human decomposition products in soil can be detected in the lab with Mid-Infrared (MIR) spectroscopy and in the field with Near-Infrared (NIR) spectroscopy; and (2) how the stage of decomposition, indicative of the Postmortem Interval (PMI), can be estimated from the spectra.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing homicide investigators with a method of detection and characterization of human remains located either on the soil surface or within clandestine burials.

After death, the neutral fats of adipose tissue decompose to fatty acids, both saturated and unsaturated. Over time, the unsaturated fatty acids are converted first to saturated fatty acids and eventually to insoluble salts that are relatively immobile and long lasting. These fatty acids and their salts contain long carbon chains consisting primarily of methylene groups (-CH<sub>2</sub>-) that have characteristic features in the infrared spectrum, both in the MIR and NIR regions. Buried human remains decompose under largely anaerobic conditions, where the decomposition process is relatively slow. Human remains on the ground surface are in aerobic conditions, and therefore decomposition of cadavers on the surface proceeds more quickly.

This study acquired spectra of several different types of soil samples in order to determine how MIR and NIR spectra can be used to detect these fatty acids in the soil: (1) soil with added saturated and unsaturated fatty acids of known concentrations, (2) soil from a mock crime scene using a pig as a proxy for a human cadaver in Ohio, and (3) soil taken from the vicinity of four decomposing human cadavers, on the surface and in burials, at the Facility for Outdoor Research and Training (FORT) in the Institute of Forensic Anthropology and Applied Sciences at University of South Florida. The presence of human remains was successfully detected in the soil samples in both spectral regions, MIR and NIR. In addition, it was demonstrated that fatty acid concentrations varied depending upon the region of the cadaver (e.g., head, pelvis, torso, feet) and the depth and distance from a cadaver that a sample was taken. In the MIR, the spectroscopic signatures of saturated and unsaturated fatty acids are distinguishable, yielding information about the extent of the decomposition and hence about the PMI. Measurements on background soil samples indicate a very small fatty acid background (~3%), not attributable to the presence of a cadaver. This background signal has a characteristic spectral signature that can be distinguished from the burial remains. The characteristic fatty acid peaks in the NIR represent the overtones of the absorptions of the fundamental vibrations in the MIR, and thus are very much less intense. Nonetheless, these weaker NIR peaks have been observed for each cadaver for which MIR peaks were observed. The advantage of the NIR measurement is the possibility of *in situ* measurements in the field. Such field measurements employ a spectroscopic probe that is pushed into the soil and connected to spectrometers on the surface by fiber optics.<sup>1</sup> Thus, these spectroscopic methods can be used in the field for the detection and characterization of clandestine human burials.

### Reference(s):

1. Matney T., Barrett L., Dawadi M.B., Maki D., Maxton C., Perry D.S., Roper D.C., Somers L., Whitman L.G. *In situ* shallow subsurface reflectance spectroscopy of archaeological soils and features: A case-study of two Native American settlement sites in Kansas, *J. Archaeol. Sci.* 2014; 43:315-324.

### Human Decomposition Product, Infrared Spectroscopy, Clandestine Burial



### A93 Geographic Information Software (GIS) Application for Building a Nationally Representative Forensic Taphonomy Database

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**Learning Overview:** After attending this presentation, attendees will understand the implications of the lack of a robust dataset for examining and improving methods to determine the Postmortem Interval (PMI) in forensic investigations. Attendees will learn about a new application, geoFOR, which will be used to address this gap in the research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a novel mechanism to collect data about decomposition using a crowdsourced data model. This presentation will introduce a new application, geoFOR, for creating a reference database of decomposition and environmental variables from which improved methods from determining PMI can be developed.

PMI determination is a critical piece of information to determine when human remains are discovered. An accurate determination of PMI can facilitate the identification of an unknown individual and help to reconstruct the events around the time of death. A major weakness with the current state of the research is the lack of a reference dataset with a large number of cases from which research questions regarding factors impacting the rate of decomposition can be addressed.

This presentation will be used to demonstrate an application that can be used by practitioners using crowdsourced data to collect information from scenes where human remains are found. This research utilizes a spatially coded, Geographic Information Systems (GIS) application that is accessible from mobile devices and tablets, among other devices. Forensic investigators working on a case use the app to record basic scoring information on the state of decomposition, upload photos of the state of decomposition, and the GIS software records the location of the discovery. The application will be available for use by investigators from across the country and internationally in order to develop a large reference sample that will be used to create improved models for determining PMI.

Much of the PMI research has focused on a few outdoor research facilities. These are geographically circumscribed areas that have a limited range of environment factors that characterize the variables known to impact the rate of decomposition. The other major source of data for PMI methods are retrospective case studies that are based on small sample sizes and are regionally specific. A review of recently published research articles demonstrates the dependence on small samples for modeling the complex decomposition process.

The decomposition process is influenced by a wide range of factors, both intrinsic to the individual and extrinsic environmental variables. The only way to develop accurate models of this complex system is to have sufficiently large sample sizes. In the absence of a large dataset, a scientific investigation of PMI is severely limited. The small sample sizes and dependence on case studies has limited research in PMI. Previous research has demonstrated that there are multiple factors that impact the rate of decomposition, and we cannot hope to develop a robust and multifaceted model in the absence of a representative and large dataset. In order for the field to move forward and to develop models that are reliable, useful, and with known error rates, a large dataset is necessary and currently missing from the field. The forensic community can work together to construct a reference set to build models of decomposition and improving methods for determining PMI when remains are discovered.

#### GIS, PMI, Database



## A94 Why Hasn't the United Kingdom Got a Human Taphonomy Facility (HTF) Yet?

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**Learning Overview:** After attending this presentation, attendees will have gained an understanding of the issues surrounding the complex and controversial campaign to establish an HTF in the United Kingdom. Attendees will learn about the legal situation and the objections that have been raised from different communities and will hear the arguments for and against HTFs, particularly in the United Kingdom, from scientific, ethical, moral, and religious standpoints. Attendees will hear about positive and negative media publicity surrounding the venture and how this has affected public opinion of the venture.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by giving the current United Kingdom perspective on the HTFs that have been so readily established in other countries and by explaining why the United Kingdom has not followed suit yet. This has important implications for forensic science internationally, as the United Kingdom may draw on and learn from the experiences and expertise of the existing facilities in terms of informing forensic cases and engaging the public.

HTFs, or “Body Farms,” are outdoor laboratories where scientific research is conducted on donated human cadavers in order to understand how human decomposition progresses in a variety of conditions. There are currently eight such facilities in the United States, one in Canada, one in Australia, and a “Forensic Cemetery” in mainland Europe.

Currently, in the United Kingdom, empirical decomposition research is conducted using animal analogs (mainly *Sus scrofa*), and university-based Animal Taphonomy Facilities (ATFs). However, concerns have recently been raised about the use of animal remains as human analogs in forensic research.<sup>1-3</sup> There is recognition that HTFs offer the chance to study the effect of certain human conditions and lifestyle choices on decomposition rate and subsequent postmortem interval estimation; something that is not possible to do with animal analogs.<sup>4</sup> This, coupled with the ever-demanding requirements needed of the expert witness in court, including possessing relevant expertise and providing reliable evidence, means that the United Kingdom is falling behind the rest of the world. So, United Kingdom forensic scientists have started to ask the question “Why hasn't the United Kingdom got an HTF yet?”

The possibility of opening an experimental facility is a dramatic and controversial proposal for the United Kingdom, for a variety of reasons. There are objections on scientific grounds, mainly based on concerns about scientific rigor and reproducibility.<sup>5</sup> There is also ethical, moral, and religious opposition, as well as substantial legal obstacles.<sup>6,7</sup> In addition, there is public opinion and perception to consider. There has been a lot in the media recently about this possibility, and it has both garnered support and provoked objections from the public.<sup>8</sup>

This presentation will discuss the current challenges and impediments to the establishment of an HTF in the United Kingdom, the scientific, legal, ethical, and moral objections, and the cogent arguments for and against one in the United Kingdom. It will discuss the risks associated with the creation, delivery, and day-to-day running of a facility. It will show how the United Kingdom situation differs from other countries where HTFs exist, and explore what the United Kingdom can learn from their experiences.

This presentation will also discuss current public opinion in the United Kingdom, based on the results a recent online survey conducted to determine the extent of acceptability of the concept of a HTF among members of the public in the United Kingdom and abroad. This presentation will present an analysis of the respondents' ( $n=600$ ) views toward different aspects of HTF operation and determine the key areas of support and objection toward a HTF being proposed in the United Kingdom.

### Reference(s):

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### Human Taphonomy Facility, United Kingdom, Public Opinion



### A95 Changes in the Cadaver Decomposition Island (CDI) and Soil Chemistry: An 18-Month Time Series for Scavenged and Protected Human Cadavers

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**Learning Overview:** After attending this presentation, attendees will have a clear understanding of what a surface human CDI looks like with and without scavenging interference and soil chemical composition results obtained throughout the study. Attendees will understand that the CDI is present months after the remains are removed and the concentration of soil nutrients fluctuate during the decomposition process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing awareness to those entities participating in search and rescue efforts to not only focus on looking for the skeletal remains of an individual, but to also look for the CDI and collect soil samples at appropriate depths.

Both the CDI and soil nutrient composition are useful pieces of evidence. Typically, during the late phase of the Early stage of human decomposition, fluids of the body, formed during autolysis, hydrolysis, and by other chemical and bacterial actions, leach into the soil under and around the body, producing a CDI. The fluid contains high concentrations of organic matter and microorganisms, eradicating any vegetation adjacent to and under the body. When the soil pH and nutrients in the upper soil horizons appear to return to a level similar to the original state, translocated nutrients in the soil cause native vegetation, and flora not indigenous to that area, to flourish. In August 2018, five cadavers were placed and the CDIs were monitored monthly for vegetation regrowth. Soil samples were taken to a depth of 40cm (in 10cm increments) after purge, then monthly thereafter for a period of 18 months. This study was conducted at the Applied Anatomical Research Center (AARC) in Huntsville, TX.

The study demonstrated that the CDI is greatest under the trunk, reduced under the head, and further reduced under the limbs. The study documents the timing of the formation of the CDI as well as the timing of the return of vegetation around and within the CDI. After collapse of body cavities and formation of the CDI, caged subjects were removed from their CDI at predetermined intervals of zero, two, and four weeks after purging. Cages remained over the CDIs and daily observation occurred until regrowth within the CDI formed. Most of the CDI for subjects accessible to scavenging had formed under the trunk of the body prior to being moved around by scavengers. However, the CDI of the limbs and head could not be clearly identified after movement of the body by scavenging had smeared the soil. Bodies were pulled away from the major part of the CDI by scavengers, but remained at the periphery throughout the study. The trunks of the bodies that had minimal movement by scavenging prolonged the CDI's soil nutrient composition and evidence of nutrient translocation to deeper depths was observed. In search and rescue efforts for missing individuals presumed dead, a CDI may be detectable a minimum of 12 months after death, even if the body has been displaced from its original location. CDI observation and soil chemistry results will be presented.

#### CDI, Soil Chemistry, Scavenged

## A96 The Importance of Taphonomic History When Conducting Histological and Isotopic Analyses on Bone

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how taphonomic histories influence microscopic and isotopic composition of bone.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting how differences in taphonomic history can be observed histologically as well as impact the isotopic composition of bone.

The 1943 Battle of Tarawa resulted in more than 5,000 casualties on Betio Island. The requisite need to bury the casualties immediately after the battle and the post-war repatriation efforts in the late 1940s led to commingling and disassociation of remains. Through a variety of anthropological methods, the Defense POW/MIA Accounting Agency has re-associated recent field recoveries from Betio Island with individuals that were buried at the National Memorial Cemetery of the Pacific (NMCP) in 1949 as “Unknowns.” The Unknowns were disinterred from the NMCP between 2016 and 2017.<sup>1,2</sup> The Unknowns were treated with formaldehyde, while the skeletal elements that remained on Betio Island were not. The re-association of recent field recoveries with disinterred Unknowns allowed for a direct comparison of the same individual skeletal elements with vastly different taphonomic histories. The purpose of this study was two-fold: first, to determine if taphonomic history could be indicated through histology and, second, to determine if taphonomic history degrades isotopic analyses.<sup>3</sup>

A total of 18 skeletal elements, representing nine antimeres or fragment refits from five individuals, were compared histologically using normal and circular polarized light microscopy. Thin sections were prepared following standard histological protocols. The extent of diagenetic alteration of the bone microstructure was scored following the Oxford Histological Index (OHI); intensity of birefringence, extent of cracking, presence of discoloration, and the type of microbial alteration were also recorded.<sup>4</sup> Ten skeletal samples—two per individual, representing antimeres and fragment refits from field and NMCP contexts—were prepared for isotope testing as collagen and bioapatite at California State University, Chico. Collagen was submitted to the Stable Isotope Facility at the University of California, Davis for measurement of carbon and nitrogen isotope values ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively). Bioapatite was submitted to IsoForensics, Inc. for measurement of  $\delta^{13}\text{C}$  and oxygen ( $\delta^{18}\text{O}$ ) values.

The taphonomic history of the skeletal elements was reflected in characteristic alterations of the bone microstructure. Remains from Betio Island showed a greater extent of diagenetic alteration (bioerosion) in comparison to those treated with formaldehyde. Despite this, the amount (yield) of collagen extracted for isotope testing was consistently higher for elements from Betio Island as opposed to treated bone. Three of the five NMCP samples had collagen yields lower than the accepted 5% threshold, while one of five samples from the NMCP had a bioapatite yield lower than the accepted 25% threshold.<sup>5</sup> Considering all samples, regardless of material yield, mean pairwise differences in collagen  $\delta^{13}\text{C}$ , collagen  $\delta^{15}\text{N}$ , or bioapatite  $\delta^{13}\text{C}$  values were not significant when compared to a theoretical mean of 0. There was a significant difference for bioapatite  $\delta^{18}\text{O}$  values (one-sample *t*-test;  $p=0.0002$ ), with higher  $\delta^{18}\text{O}$  values measured for samples from Betio Island.

This research has implications for biomolecular forensic analyses of chemically altered remains (e.g., embalmed, prepared anatomical materials, etc.). Disinterred samples previously treated with formaldehyde demonstrated well-preserved bone microstructure due to the biocidal effects of the preservative, but overall lower collagen yields. Based on yield data, isotope testing results of 60% of prepared collagen samples could be considered potentially suspect. Conversely, samples from recent Betio Island recoveries demonstrated poor preservation of bone microstructure yet yielded reasonable amounts of both collagen and bioapatite (with one exception) for isotope testing. Disregarding material yields, collagen from disinterred bone was not isotopically distinct from collagen from recent Betio Island-recovered bone when considering either carbon or nitrogen isotopes values, while only  $\delta^{18}\text{O}$  values (and not  $\delta^{13}\text{C}$  values) of bioapatite differed between paired samples. The taphonomic history of a bone, like embalming or burial environment, influences the analytical results derived from that bone and thus should be considered during forensic analyses.

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### Taphonomy, Histology, Isotopes

## A97 Prediction of Minimum Postmortem Submersion Interval (PMSI<sub>min</sub>) Based on Eukaryotic Community Succession on Skeletal Remains Recovered From an Aquatic Environment

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**Learning Overview:** After attending this presentation, attendees will have acquired information on a new method designed to aid in the determination of PMSI<sub>min</sub>, specifically from waterlogged bones.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees how the results obtained from this research may be applied to determine time of death for remains found in cold cases, which may corroborate eyewitness testimony, as well as increase accuracy in the estimation of PMSI in combination with bacterial succession.

Many recent studies were conducted concerning bacterial succession in decomposing animal carrion in terrestrial systems.<sup>1,2</sup> However, not much is known about the microorganisms involved in aquatic decomposition.<sup>3</sup> Dickson et al. presented a study on marine bacterial succession to determine PMSI using partial pig remains; however, this study did not utilize next-generation sequencing technologies.<sup>3</sup> Per research, there are currently no published studies which estimate PMSI based on eukaryotic community succession in aquatic systems. The main goal of this study was to determine the eukaryotic community succession associated with porcine skeletal remains in lentic aquatic environment and to derive a statistical model for PMSI<sub>min</sub> prediction.

Henleys Lake in Crozet, VA, was the location chosen for this research. Fresh pig bones (rib  $N=100$ , scapula  $N=100$ ) were placed in cages (10x10 inch<sup>2</sup>), attached to a floatation device, and submerged using waterproof loggers (to record hourly temperature), and a Yellow Springs, OH Sonde™ (to monitor pH, specific conductivity, dissolved oxygen, salinity, and depth). Every 250 Accumulated Degree Days (ADD), one cage containing five rib and five scapula samples was collected, photographed, and stored at -80°C until processed. Water samples were also collected every 250 ADD and filtered using a cellulose membrane filtration system. DNA extraction was performed using the Invitrogen® ChargeSwitch® gDNA Plant Kit Protocol. Variable region nine (V9) of the 18S rRNA gene was amplified and sequenced using the primers as described by Earth Microbiome Project, including a mammalian blocking primer, and was based on dual-index strategy as described in Kozich et al on MiSeq® FGX sequencing platform.<sup>4</sup> Sequenced data were quality controlled and analyzed via the MiSeq® SOP in Mothur version 1.36.<sup>4</sup> Hierarchical classification of good quality sequences was performed based on SILVA119 database. A phylogenetic approach was utilized for  $\alpha$ - and  $\beta$ -diversity estimation. Analysis of Molecular Variance (AMOVA) was used to test statistical differences in eukaryotic colonization of bone types over ADD.

Preliminary results at the Phylum level (level 2) showed that eukaryotic community associated with rib and scapula samples were similar, characterized by the combined presence of Ciliophora, Ochrophyta, and Peronosporomycetes. Scapula samples showed a difference due to the added presence of Euglenozoa (2.1%). The eukaryotic community changed significantly with ADD in both bone types ( $p < 0.0002$ ). Samples collected at 250 ADD were characterized by the presence of Peronosporomycetes (74.3%) and Dinoflagellata (1.9%, found highest at this time point). The 500 ADD collection was characterized by the presence of Peronosporomycetes (90.4%). Peronosporomycetes abundance declined from 750 ADD through 3,250 ADD, after which an increase was observed (e.g., 51.3% at 4,250 ADD). Collections spanning the 1,000 ADD to 3,250 ADD interval were characterized by Ciliophora (>20%), and the final collection (4,750 ADD) was categorized by the combined presence of Ciliophora (14.7%), Euglenozoa (3.9%), and Peronosporomycetes (6.8%).

In conclusion, this study highlights the eukaryotic community associated with bones in lentic systems and, in the future, this information may be utilized for the development of statistical models for prediction of PMSI<sub>min</sub> either alone or in combination with bacterial community succession.

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### PMSI, 18S rDNA Sequencing, ADD



### A98 Quantifying Missing Migrant Antemortem Data and Their Identification Significance

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**Learning Overview:** After attending this presentation, attendees will better understand the complexities of identifying remains of missing migrants from poor, primarily rural, underserved communities in the Central America-Mexico-United States migrant corridor, as well as be better informed regarding the probability of obtaining significant antemortem data for achieving identifications in these cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing quantified analysis of antemortem data on missing migrants from the Central America-Mexico-United States migrant corridor that can aid practitioners in the design of effective antemortem data collection tools, help them better understand the value of an antemortem feature coincidence, and inform their assessment of the prevalence of different methods as part of a multidisciplinary identification.

This research project works to further define and illuminate the value of antemortem data in the multidisciplinary forensic investigation process through a case data-based analysis. The research introduced in this presentation seeks to further delineate types of antemortem data useful in forensic investigations into missing migrant cases in the Central America-Mexico-US migration corridor, examining what can be considered significant antemortem data in this regional context by evaluating which types of antemortem data have been forensically significant in supporting identifications for a multi-year, transnational project, the Border Project (Proyecto Frontera).

The Border Project is an initiative that aims to create a transnational mechanism for exchanging forensic information on missing persons and unidentified remains to significantly improve both the identification of missing migrants among unidentified remains in the region and the response of governments to families searching for missing migrant relatives. In this presentation, an analysis of the aggregated dataset from these cases will be presented, encompassing investigations launched since 2010 into missing migrant cases dating from 2000 through to the present. This dataset includes more than 1,300 cases of missing migrants coming from multiple countries of origin in Central America, as well as Mexico, who have gone missing in Mexico and the United States.

When working with cases of human rights violations and missing migrants in Latin America, antemortem data can tend to be limited in terms of formal, recorded documentation, such as medical records, dental records, and fingerprints, due to multiple socioeconomic factors. The vast majority of these cases do not have official medical or dental records, as missing individuals are more likely to have received such care in highly localized circumstances in which no records may be available or are not retained. Thus, antemortem data in the majority of these cases are based on the memories of relatives of the missing person. Nonetheless, such antemortem data have been shown to be highly useful in Border Project missing migrant cases, both in the investigation process and as a tool for exclusions of inconclusive initial genetic matches. This presentation will introduce the context and challenges of reaching identifications in this corridor and will describe each of the antemortem features evaluated in the quantitative analysis. The conclusions provided in this presentation will highlight the antemortem data types that have proven to be most significant in the investigations to date and discuss the evaluation of their level of significance. This presentation will also present differences in antemortem data significance between different countries of missing person case origin in Central America and Mexico, which also may change over time, due to factors such as the local availability of specific dental restorations or the prevalence of tattoos.

A multidisciplinary, integrated approach to the identification of remains is always important, as each context in which a person goes missing—each country, time period, incident, etc.—can affect which elements are more available or more important to prioritize in an investigation. In different circumstances, different factors in reaching an identification may take a lead role in the investigation, including genetics, fingerprints, and antemortem data. Quantifying antemortem data and their forensic significance in reaching identifications for a specific regional context helps to define the leading data factors that can aid in the identification process, thereby allowing more time and resources to be dedicated to those areas of data collection. Moreover, better understandings of the antemortem data that hold the greatest significance in missing migrants case investigations can help to define effective public policy and support establishing protocols and tools that focus on collection of the most impactful information for investigators.

#### Antemortem, Quantification, Missing Migrants



### A99 DNA Identifications of Migrant Remains at the Pima County, AZ, Office of the Medical Examiner (2000–2019)

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**Learning Overview:** After attending this presentation, attendees will understand the methods used by one border medicolegal office to achieve more than 400 genetic identifications for Undocumented Border Crossers (UBCs).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a successful model for the facilitation of genetic identifications of missing migrants that is achieved through the rigorous maintenance of data and close inter-agency collaborations.

The Pima County Office of the Medical Examiner (PCOME) implements several methods to aid in the positive identification of the more than 100 unidentified UBCs reported to the Office every year. These methods include fingerprints, visual identifications, and DNA comparisons, among several others. The PCOME has a steady identification rate of approximately 65% of cases, with a trend toward DNA identifications becoming more prevalent over the past eight years. More than 400 individuals have been scientifically identified using DNA analyses since 2000, despite funding issues that resulted in a multi-year backlog of a large proportion of unidentified cases. A 2018 National Institute of Justice (NIJ) Coverdell Award has resulted in the necessary funding to resolve this backlog, with additional identifications expected in the fall of 2019.

Given the circumstances surrounding the majority of unidentified individuals examined at the PCOME, primarily their undocumented and foreign national status, a multitude of resources have been used to aid in their identification. Best efforts are made by the PCOME with local and national law enforcement to establish identifications in the most rapid and cost-effective manner possible, but in many cases DNA analysis becomes the only available option. Behind fingerprints, genetic comparisons are the second-most-frequent method of scientific positive identification used at the PCOME and have accounted for roughly 21% of identifications. As of mid-2019, more than 1,200 samples have been analyzed by Bode Technology™, and an additional 176 are in the beginning stages of analysis; other laboratories, including domestic and international partners, have processed more than 300 PCOME cases. To date, 401 individuals have been scientifically identified using DNA profiles developed by at least four domestic laboratories and through the Mexican Federal Police. The majority of genetic identifications for UBCs (354/401) were established through DNA comparisons at Bode Technology™, while government labs have accounted for slightly more than 10%.

Whether the genetic comparisons take place at a private lab or government lab, the PCOME depends on close partnerships with local law enforcement, Non-Governmental Organizations (NGOs), and foreign consulates to support the collection of missing person information and Family Reference Samples (FRS). Among consulates, the PCOME works most regularly with the Tucson, AZ, office of the Mexican Consulate, which has supported the identification of 325 of the 401 cases, either through funding DNA collection and comparison, providing antemortem data, or completing information needed for case closure and death certificate completion, or all of the above. Similarly, the Guatemalan Consulate has supported 46 such cases, the Honduran Consulate 16, and the Consulate of El Salvador with 10 cases.

In addition to the support from foreign consulates, the PCOME depends on close collaboration with two key NGOs, the Colibrí Center for Human Rights, and the Argentine Forensic Anthropology Team (EAAF). Both NGOs provide families of missing migrants with a safe and secure mechanism to report missing loved ones and to submit DNA for the purposes of comparison against unidentified remains examined at the PCOME. As of the summer of 2019, the Colibrí Center for Human Rights has facilitated 48 genetic identifications and the Argentine Forensic Anthropology Team with 50 in collaboration with the PCOME. All such identifications include multi-agency partnerships, with consulates providing the necessary governmental link to ensure families are able to repatriate remains to their home countries. Some successful identifications included both Colibrí and EAAF, with one NGO collecting family reference samples in a United States state, and the other within the family's country of origin.

The prolonged duration and wide geographic scope of the international crisis that has resulted in the PCOME undertaking the identification of close to 2,000 UBCs has necessitated flexibility and creativity. In this context, the power of the science and technology of DNA can only be realized through international collaboration and public-private partnerships.

#### Undocumented Border Crossers, DNA Identification, Border Issues



### A100 Investigating Unidentified Migrant Remains Across Texas Jurisdictions: An Analysis of Positive Identifications by Operation Identification at Texas State University

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the positive identifications of unidentified migrants made by Operation Identification (OpID) at Texas State University, the time it takes to make such identifications, and need for centralized investigative efforts in Texas.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of OpID's investigative activity as well as promoting best practices and strategies for improving identification outcomes of unidentified remains across Texas jurisdictions.

In 2012, Texas surpassed Arizona as the state with the highest rates of migrant death. According to United States Customs and Border Patrol statistics, a total of 1,566 migrant deaths occurred in Texas from 2012 to 2018, though more than 4,000 migrant individuals have died in the state since 1998. Whereas Arizona has the centralized agencies of the Pima County Office of the Medical Examiner (PCOME) and the Maricopa County Office of the Medical Examiner (MCOME) to investigate the deaths of most unidentified migrants recovered from the state's public land, investigations in Texas are county dependent, with most cases being handled by justices of the peace. Because only 14 of the 254 counties in Texas have medical examiner's offices, forensic experts are often not involved in the investigation of unidentified migrant deaths, despite statewide legislation that all unidentified remains undergo inquests and DNA sampling. The need for increased forensic resources and streamlined investigative systems in Texas is urgent.

OpID, founded in 2013, seeks to recover the remains of unidentified migrants found along or near the United States-Mexico border in order to facilitate positive identification and repatriation. To date, OpID has 287 active unidentified remains that have been recovered from ongoing cemetery exhumation efforts or transferred from other medicolegal agencies or law enforcement authorities. Since 2013, OpID has made a total of 35 positive identifications. Despite the much-needed answers that these identifications have given families, the identification process for undocumented migrants still remains challenging, in large part due to the fragmented nature of death investigation in the region. In addition to the difficulty of recovering well-preserved remains from vast expanses of private land, the burial of unidentified migrant remains without proper sampling or documentation greatly hinders their ability to be identified when they are exhumed.

The goal of the current presentation is to examine all of the positive identifications made by OpID in order to shed light on the timing, obstacles, and potential solutions inherent to investigating unidentified migrant death in South Texas. All positive identifications made to date by OpID were analyzed in relation to case demographics, recovery location, and the identification process undertaken, including the timing from recovery to positive identification, the means by which cases were solved (i.e., DNA or fingerprints), and the degree of non-OpID forensic practitioner involvement. For all OpID-positive identifications, the average time from recovery to identification was just over three years (3.3 years), with cases ranging from two months to nearly seven years. OpID cases that had been recovered from exhumation efforts took on average two years longer than cases that had been transferred to OpID from medical examiner or local authorities' offices, though the timing for these transfer cases was also slow (an average of 3.9 years and 1.9 years, respectively). Case studies will be presented to further discuss the timelines of these investigations.

Solving unidentified migrant cases in Texas requires the centralization of postmortem data and extensive coordination between many agencies. While the average time to identify remains is just over three years, there are still more than 200 individuals that have yet to be identified. Data sharing and case coordination between local authorities, forensic institutions, and international stakeholders could aid in the investigation of these cases and shorten the timeline. However, this degree of cooperation needed also requires policies, funding, and educational outreach to ensure compliance with state law. While exhumations of the long-term dead will still be necessary to make positive identifications, future cases should not be interred without proper analyses, sampling, and documentation from forensic practitioners.

#### Migrant Deaths, Identifications, Human Rights



### A101 The Application of Geographic Information Systems (GIS) to the Migrant Crisis in South Texas: Modeling Migration in Brooks County, Texas

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**Learning Overview:** After attending this presentation, attendees will understand the benefit of applying GIS to evaluate the movement of migrants through the South Texas landscape.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing on search strategies for missing migrants, which will increase recovery rates and aid in optimizing the placement of water stations throughout the landscape.

Current immigration policy and border enforcement strategies have contributed to an ongoing humanitarian crisis at the United States-Mexico border, as people continue to migrate north fleeing poverty and violence. As migration from Central America surged, Texas surpassed Arizona in migrant deaths, resulting in thousands of deaths and unidentified human remains recovered across the South Texas landscape.<sup>1</sup> Much of the current recovery and identification efforts in Texas have centered around Brooks County, where more than 600 presumed migrants have been recovered from 2009 to the present. However, missing persons records largely outnumber unidentified recoveries, indicating that many of the missing have not been found. This mismatch is likely because approximately 96% of Texas is private land, preventing any large-scale searches for migrant remains.<sup>1</sup> Thus, many deceased migrants are found through happenstance. The present research attempts to model migrant movement across Brooks County to better understand where deceased migrants may be found. By incorporating known migrant death locations into the model, this project seeks to improve current search efforts, recovery rates, and the placement of water stations.

Two migration models (Model A and Model B) for Brooks County were generated using ArcGIS® v10.6.1.<sup>2</sup> Cost surface variables include slope, jaggedness, and land use data from the United States Geological Survey (USGS).<sup>3,4</sup> Model A incorporates a cost surface where developed open space is designated as low cost, and all other land use codes are designated as high cost. This surface intends to route migrants through the county by only utilizing open areas, including rural roads and open grassland, and avoiding any moderate to dense vegetation or development. Model A serves as a proxy for easiest movement across the landscape. Model B incorporates a more complex cost surface where cost values are assigned to land use codes based on a theorized compromise between ease of movement and avoidance of detection. Although developed open space is still considered less costly in Model B, shrub/scrub land codes are assigned moderate cost values as it makes moving through the landscape more difficult, while providing camouflage to prevent detection during migration. Two hundred fifty Least Cost Paths (LCPs) were generated from 10 origin points along the southern border of Brooks County to 25 destination points placed along a known pickup road (HWY 285) in the north of the county. Four hundred thirty-seven death locations, obtained from Brooks County Sheriff's Office recovery reports, were then compared to the LCPs from each model.

Near distance analysis of death locations to LCPs suggest Model B more closely approximates how migrants are moving through the landscape in Brooks County when compared to Model A, as indicated by a shorter near distance mean and standard deviation. However, Model A and Model B produce significantly farther distances between death locations and LCPs than when death locations are measured directly to any developed open space code from the original USGS data for the county.

These results suggest that migrant movement across the South Texas landscape is complex, particularly as migrants become dehydrated and disoriented, and that recorded death locations are skewed toward frequently traversed access routes. Continuing research will incorporate hotspot analysis and water station placement to better understand how migrants are interacting with the surrounding landscape. The results of this research can be used by search and recovery teams to develop feasible and efficient strategies that target areas in Brooks County where deaths are more likely to occur. Furthermore, the models that best approximate how migrants are moving through the landscape can be applied to surrounding border counties in an effort to locate and recover more migrant remains.

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#### Global Information Systems (GIS), Migrants, Least Cost Path Analysis





## A102 NamUs Location Data: Cluster Analysis of Migrant Deaths at the Texas-Mexico Border From 1990 to 2017

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**Learning Overview:** The goal of this presentation is to identify significant spatiotemporal clustering of migrant deaths along the Texas-Mexico border with SaTScan™ software, direct search and recovery efforts based on those clusters, and assess how informative the location data reported on the National Missing and Unidentified Persons System (NamUs) is.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an example of the application of SaTScan™ and ArcGIS® software utilizing Space-Time Scan Statistics (STSS) to detect migrant mortality clusters and assess the accuracy of publicly available locational data from NamUs.

Migrant deaths in Texas drastically increased in 2012, eclipsing the other United States-Mexico border states, and remain high to this day.<sup>1</sup> Local law enforcement, medical examiner's offices and contract pathologists, academic and humanitarian institutions, and United States Customs and Border Patrol (USCBP) face unique challenges in locating and recovering these remains. Specifically, a majority of land in Texas is privately owned (over 95%) and therefore not regularly patrolled by local law enforcement without permission by landowners.<sup>1</sup> Further, there is not systematic collaboration in search and recovery practices because jurisdiction is divided along county lines, of which there are 254 in Texas.<sup>1</sup>

Implementing spatiotemporal analyses of migrant death recovery sites could overcome the challenges to search and recovery efforts ongoing in Texas. STSS can direct efforts by indicating geographic and/or seasonal patterns in death clusters. The goal of this research is to detect significant spatiotemporal clustering of unidentified deceased migrants found along the Texas-Mexico border between 1990 and 2017.

**Methods:** A research area of 31 Texas counties was defined, in which the county was along the border or contained a USCBP check point, constituting ostensibly a second border.<sup>1</sup> NamUs case report data dating from 1990 to 2017 were obtained from these counties where the case notes indicated the unidentified decedent was a probable migrant (i.e., physical description, anthropological analysis, or recovery context).<sup>2</sup> These parameters yielded a total of 659 cases.

Locational data was derived from three different fields within the Circumstances section of each of the reports: (1) auto-generated coordinates through a Google® Maps link, (2) Global Positioning System (GPS) coordinates directly entered by a case manager, or (3) county centroid coordinates based on the location remains were found. All GPS coordinates were converted into decimal degrees for consistency. ArcGIS® 10.6.1 was used to calculate the centroid coordinates.<sup>2</sup> A file was prepared with ArcGIS® 10.6.1 containing case, date found, and location data for analysis in SaTScan™ v.9.6.<sup>2,3</sup>

SaTScan™ performs STSS with a 3D circular window, the base of the window representing the geographic space and the height corresponding to time.<sup>3</sup> SaTScan™ identifies significant spatiotemporal clusters in the Texas border region if, for a specific time period, that area has a higher proportion of migrant cases compared to the remaining geographical areas.<sup>3</sup> The spatial window size was set to not exceed 10% of the study area to ensure smaller cluster phenomena could be detected as well as indicate temporal trends, such as clustering in particular seasons.

**Results/Conclusions:** Significant spatiotemporal clusters were found with Google® Maps and county centroid coordinates, specifically centering near West Texas around El Paso County, Southwest Texas around Maverick county, and South Texas around Brooks county. However, identifying meaningful clusters was contingent on the accuracy of location data available through NamUs. The location data was of mixed scale and therefore cases were not necessarily comparable when trying to identify areas with high mortality incidences. None of the significant clusters' dates corresponded to a season or was narrow enough to be associated with USCBP border securitization activity. No significant clustering was detected using case manager's GPS coordinates, which was likely due to relatively few data being available ( $n=75$ ). The clusters are more likely indicative of documentation behavior by local law enforcement, USCBP, medical examiners, or contract forensic pathologists in the region.

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### NamUs, SaTScan, Cluster Analysis



### **A103 Operational and Leadership Assessment During the Puerto Rico Forensic Science Project: How the American Society of Crime Laboratory Directors (ASCLD) Lab Directors, National Association of Attorneys General (NAAG) and Forensic Scientists Assisted the Puerto Rico Laboratory After Hurricane Maria**

*Matthew J. Gamette, MS\*, Idaho State Police Forensic Services, Meridian, ID 83642; Amie Ely, JD\*, National Association of Attorneys General, Washington, DC 20036*

**Learning Overview:** After attending this presentation, attendees will understand the operational and logistical challenges a forensic laboratory may face after a massive natural disaster or significant economic catastrophe. Attendees will be asked to consider the “cracks” or risks in their own laboratories that may be exposed with a significant stressor such as a natural disaster or financial crisis. Attendees will take away some considerations when engaging in benevolent volunteerism as an individual or organization. Attendees will learn about the struggles of an amazing group of colleagues in Puerto Rico and an inspiring group of volunteers from the continental United States that have activated to make a difficult situation better for their colleagues.

**Impact on the Forensic Science Community:** “Labs Helping Labs” is an initiative that needs to continue to grow. Forensics is a unique community where culturally colleagues want to help each other. It is a unique community because forensic science volunteers have a special skills set that must come from the trained experts in the forensic science community. As the number of natural disasters and the amount of political instability increases in the world, forensic scientists must be prepared to immediately deploy the necessary help to bolster the criminal justice services to impacted communities. Critical lessons learned from this project can provide a head start in emergency preparedness and volunteerism efforts all over the world.

In April 2018, ASCLD was asked by the National Association of Attorneys General (NAAG) to provide an operational needs assessment of the forensic science laboratory. The initial needs assessment included an evaluation of facility damage due to Hurricane Maria, instrument damage and other factors inhibiting forensic work, and backlogs existing before and after Hurricane Maria. After discussing the forensic science laboratory critical needs, meetings were held with federal government entities, Puerto Rican government officials, forensic laboratories around the country, and many forensic science instrument and service vendors to coordinate a response. The ASCLD President and the ASCLD Board determined resources the organization could provide and continued to partner with NAAG to determine the best method to provide resources to the Puerto Rico forensic science laboratory.

On-site fact-finding visits were conducted in April and October 2018 by the ASCLD President and the NAAG Director of Center for Ethics and Public Integrity. These visits consisted of visiting with the top leadership of the laboratory, leaders of each laboratory forensic science discipline, grant managers, facility managers, evidence technicians, financial and human resources managers, customers, and stakeholders to understand the scope of issues faced by the laboratory. Immediately evident were problems with the physical facility (e.g., sustainable power, air conditioning, security), broken instrumentation, a lack of personnel, a lack of training resources, and appropriate leadership. The laboratory was in financial crisis due to federal bankruptcy imposed upon the island and also the restructuring of the Puerto Rican government.

ASCLD and NAAG established top priorities, developed a Memorandum Of Understanding (MOU) with the Puerto Rican government, and identified resources to address the most urgent issues. In May 2018, ASCLD provided scholarships for three top Puerto Rico Forensic Science Bureau (FSB) leaders to attend the ASCLD annual symposium in Atlanta, GA. In January 2019, the majority of the ASCLD Board, along with other ASCLD members, traveled to Puerto Rico to provide assistance. ASCLD taught the renowned ASCLD Leadership Academy training at the lab, and approximately 30 FSB leaders graduated. ASCLD brought experts from every forensic science discipline, quality assurance professionals, and laboratory leaders from across the country to directly engage with each technical discipline. Experts were provided a specific mission for approximately the next week to research the issues facing each discipline. Additionally, ASCLD experts were asked to perform a root cause analysis of the existing issues. ASCLD experts provided recommendations for instrument, method, personnel, and process improvement in each discipline. ASCLD appointed a project leader for addressing each forensic science discipline, quality assurance, development of a new Laboratory Information Management System (LIMS), federal grant management, financial management and purchasing, and physical facilities.

ASCLD has continued to work with FSB leaders to make necessary operational improvements at the laboratory. ASCLD experts have continued to provide direct assistance to the laboratory in countless ways. Meetings have continued in-person and via teleconferences to provide expert advice, technical assistance, and information exchange. ASCLD has facilitated donations of instrumentation and equipment, coordinated casework and technical review assistance, provided analyst training, and assisted in identifying potential volunteer and affordable vendors that could provide necessary services. ASCLD will continue to provide essential assistance for the near future as part of a new initiative titled “Labs Helping Labs.”

This presentation—which we propose will be accompanied by sister presentations by our partners at the Forensics Sciences Board (FSB), NAAG, the American Board of Forensic Anthropology, and the American Society for Clinical Pathology—will address, among other things: (1) ASCLD involvement in the Puerto Rico FSB project; (2) operational challenges the laboratory had in recovering from a natural disaster and significant government bankruptcy; (3) challenges a volunteer initiative has in working with the federal government, state or territory government, and potential vendors. Lessons learned for labs helping labs in future emergencies; and (4) lessons learned through this massive humanitarian volunteer effort, including the importance of a robust MOU, establishing connections with government entities and politicians, effective coordination of benevolent donors and avoiding potential political conflicts, addressing funding challenges to support on-site volunteerism, establishing legitimacy with disaster recovery experts and organizations, bolstering leadership and management structure amid political and financial instability, developing trust with laboratory leaders and staff in challenging circumstances, and the considerations of benevolent volunteerism versus paid contract help.

#### **Puerto Rico, Volunteer, Hurricane Recovery**



## A104 Puerto Rico Forensic Science Project: The Puerto Rico Forensic Services Perspective

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**Learning Overview:** After attending this presentation, attendees will understand the operational and logistical challenges a forensic laboratory may face after a massive natural disaster or significant economic catastrophe and key aspects in order to overcome the situation. Attendees will be asked to consider how they might provide assistance in Puerto Rico or other places that might need forensic science assistance. Attendees will be asked to consider disaster planning or recovery models that consider forensic science operations. Attendees will take away thoughts regarding how to accept help effectively in an emergency situation and will learn how critical forensic science operations are to a state or territory criminal justice process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how the critical lessons learned from this project can provide a head start in emergency preparedness and volunteerism efforts all over the world. Practitioners will learn what happens in worst-case scenario situations, and where they may be able to reach out for help. Forensic science leaders who are being asked to focus more on risk mitigation as part of the International Organization for Standardization (ISO) 17025:2017 will learn risk factors they may not be considering in their continuation of operations plans. Right now, the forensic science community is loosely organized for emergency situation response, and planners should consider the increase in natural disasters that impact forensic science service providers each year.

Puerto Rico's Negociado de Ciencias Forenses (in English, Forensic Science Bureau, or "FSB") and Puerto Rico Department of Public Safety (PR DPS) signed a Memorandum Of Understanding (MOU) with the American Society of Crime Laboratory Directors (ASCLD) and National Association of Attorneys General (NAAG) to receive professional volunteer help in recovering from Hurricane Maria. The Puerto Rico FSB has benefited in many ways from the generosity of many volunteer professional forensic scientists, laboratory directors, forensic pathologists, autopsy technicians, attorneys, and other experts from around the continental United States.

**Background of the Puerto Rico Forensic Science Project:** The FSB has experienced severe hardships in the past several years. While most are aware of a series of devastating hurricanes that hit the island, many are not aware of the government financial issues that have led to federally overseen bankruptcy of the island and Puerto Rico government restructuring. The hurricanes did millions of dollars in damage to the FSB facility, eliminated sustainable power and air conditioning to the building, and damaged a majority of the instrumentation and equipment. The Medical Examiner Office (MEO) was overwhelmed with work due to victims from and related to the hurricane, deaths from a high crime rate, and restrictive autopsy laws and policies. In addition, many of the FSB employees left the island in the wake of the hurricanes to take higher paying jobs in laboratories on the continental United States. Due to government restructuring from the bankruptcy, almost overnight FSB went from being an independently operating forensic science laboratory and MEO to being part of the newly established Department of Public Safety. FSB went through several interim Commissioners in rapid succession due to political unrest and an extreme workload. Backlogs that already existed before the hurricane increased, and new operational issues emerged that prevented the lab and MEO from meeting the needs of the criminal justice system on the island. Cases were not being worked, reports were not available for court proceedings, and grieving families could not get access to their deceased loved ones for burial. Political officials from the Puerto Rico Department of Justice, Department of Public Safety, and Governor's Office were looking for solutions to the issues at FSB.

Assistance came initially from ASCLD and NAAG volunteers, and they were able to engage volunteers from several other large forensic science organizations. While initially the FSB staff were a bit skeptical of the staying power of these volunteers, they quickly learned of the determination and resolve they brought to help the situation. They were initially able to assess operational, instrumental, and staffing needs and begin to identify and bring resources to help. They were able to gain the confidence of government officials to help FSB make assurances to those entities, the media, and accrediting bodies that the situation would improve. Efforts were made to identify and train leaders at FSB, and a new permanent Commissioner was identified and confirmed by legislative leadership. While efforts are ongoing to address physical facility, information technology, security, technical, staffing, and other operational issues, the volunteer help of colleagues from all over the United States has been extremely valuable and provides hope to the people of Puerto Rico.

This presentation—which we propose will be accompanied by sister presentations by our partners at ASCLD, NAAG, the American Board of Forensic Anthropology, and the American Society for Clinical Pathology—will address, among other things: (1) forensic science challenges in Puerto Rico before and after Hurricane Maria; (2) leadership and operational challenges the laboratory had in recovering from a natural disaster, government bankruptcy, and government restructuring; (3) challenges and benefits in accepting help from and coordinating with a volunteer initiative; and (4) lessons learned by FSB through this project, including navigating cultural and language barriers, learning to trust entities offering help and setting effective boundaries, managing and coordinating multiple organizations offering various assistance, navigating federal and state government rules and restrictions in an emergency situation, managing political, stakeholder, and public expectations without the resources, working with disaster recovery experts and organizations, and developing a leadership team and management structure amid political and financial instability.

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### **Puerto Rico, Recovery, Emergency Preparedness**



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### A105 Infrastructure, Organization, and Challenges of the Puerto Rico Medical Examiner System

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**Learning Overview:** The goals of this presentation are: (1) to describe the current structure and organization of the Puerto Rico Medical Examiner System, (2) to identify challenges in the system and their sources, and (3) to understand the impact of the suggested and implemented solutions for challenges encountered.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how all health systems can be impacted by natural disasters. In the case of Hurricane Maria in Puerto Rico, unmasked ongoing challenges in the medical examiner system that provide an excellent learning opportunity for other health systems are at risk for natural disaster.

The American Society of Clinical Pathology (ASCP), with several supporting and organizing groups, traveled to Puerto Rico on a humanitarian aid mission specifically focused on the challenges of forensic pathology in Puerto Rico. Although Hurricane Maria's path of destruction across Puerto Rico in 2017 caused major headlines for the Puerto Rico Medical Examiner System, the underlying challenges faced by the pathologists and laboratory professionals in Puerto Rico were simply unmasked and not caused by this event. The immediate deaths (direct) caused as a direct result of the hurricane required examination by the Medical Examiners (MEs) and spiked their caseload temporarily. Due to ongoing challenges across the island, additional deaths (indirect) as a result of the hurricane created controversy and challenges as attempts to measure the overall death toll became both subjectively and objectively difficult.

As is seen in many laboratories around the world and in mainland United States, staffing shortages, inefficient external processes, and resource challenges created a "perfect storm" for the ME system to come under fire. Forensic pathologists, those responsible for ascertaining medicolegal deaths, are in short supply nationally, with a significant gap in numbers of pathologists and funding for their services. Puerto Rico, likewise, has a staffing shortage that was quantified and projected by the assessment team. Like the mainland United States, the forensic services of Puerto Rico are a government-funded program and, therefore, the operations are depending on approved budgets, transparency in costing and resource allocation, and matching need with supply for all aspects of the laboratory's function. The leadership of the ME's office has made incredible strides and had documented successes in improving the funding challenges for the laboratory and continue to build out the needed resources.

With the presence of Hurricane Maria, continuous access to power, for example, became a major challenge as refrigeration is a key component of ME functionality, as well as mortuary services throughout the island. The need for temporary storage admixed with communication challenges created disinformation in the public sector. However, the core team, facilities, and processes are clearly prepared to be successful and a series of not-mutually-exclusive solutions are available to propel this system to excellence. In 2014, the ME laboratory was able to obtain the National Association of Medical Examiners (NAME) accreditation. In 2019, NAME extended provisional accreditation for the ME office, yet gaps to meeting the accreditation requirements continue and stem directly from the above challenges and not from a change in the core functionality. However, those gaps, are identifiable and amenable to sustainable changes. The experience of assessing this system, integration and modeling of data, and the presentation of solutions will be discussed.

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#### **Hurricane, Personnel Capacity, Assessment**



### A106 Decedent Tracking: A Need for an Accurate Headcount in the Medical Examiner's Office

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the need for a strong decedent tracking system, critical factors of a successful tracking system, and the need for supportive standard operating procedures. Failure to properly handle decedent inventory is a problem that many medical examiner offices have faced, including the Puerto Rico Bureau of Forensic Sciences (in Spanish, Negociado de Ciencias Forenses (NCF)).

**Impact on the Forensic Science Community:** This presentation will impact the forensic sciences community by addressing that the difficulties that NCF faced immediately and long after Hurricane Maria are not unique to Puerto Rico or to a mass disaster event. The lessons learned through evaluating the NCF decedent tracking system and developing tools that can assist with decedent handling and storage are informative to medical examiner offices throughout the world.

On September 20, 2017, Hurricane Maria, a Category 5 hurricane, made landfall in Puerto Rico, causing widespread devastation to the island's infrastructure. Initially, the death count from the storm was reported as 65 individuals; a year later, it was corrected to 2,975 individuals. The unfathomable discrepancy is an indication of the breakdown of systems that affected nearly all agencies on the island. The NCF was not immune to the devastation and experienced loss of personnel and resources that jeopardized the laboratory's accreditation and interrupted the forensic science services provided to the Puerto Rican criminal justice system. As the impact of Hurricane Maria accumulated, NCF found itself overwhelmed with decedent inventory. In January of 2019, more than a year after the hurricane, NCF required additional refrigerated trailers from the Federal Emergency Management Agency (FEMA) to handle the decedent inventory.

In 2018, the National Association of Attorneys General (NAAG) received a request from the Puerto Rico Public Safety Secretary for assistance in steadying the NCF. NAAG, in turn, requested the assistance of the American Society of Crime Laboratory Directors (ASCLD), and together these organizations began a multidisciplinary response. One component of the response was to assess the NCF system for decedent tracking and inventory. In order to understand the system, the standard operating procedures governing mortuary operations and decedent identification were reviewed, the facility was toured, and key staff members were interviewed. Decedent processing from receipt to release was mapped.

During the assessment, several obstacles to implementing a strong decedent inventory system were identified. Most notable was the use of three independent electronic data management systems, each with a slightly different purpose. None of the systems served as the master system with complete, accurate, and current information. Furthermore, none of the systems were capable of reporting a list of decedents that were received by NCF but not yet released. Decedent inventories were manually generated by staff visualizing each body bag and writing down the case number on an inventory form. This approach required a significant number of man-hours, placed staff in an uncomfortable work environment (refrigerated coolers) for a lengthy period of time, and was found to be error prone. An additional obstacle was that bodies were not examined until a family member presented to NCF and initiated the identification process. This caused significant delays in the release of the body in many cases. Finally, tentatively identified and unidentified decedents were collectively identified as John and Jane Does, putting at risk critical information needed to facilitate positive identification.

The results of the assessment (audit date April 24, 2019) indicated that NCF's decedent storage capacity, including fixed and temporary storage units, was 295 decedents. NCF was storing 269 bodies. Eighty-four of the bodies were received in 2018 or before. The longest-held body was received in 2010. Forty of the decedents were positively identified and awaiting examination. Fifty-seven of the decedents were classified as Jane or John Doe. Forty of the decedents were ready to be released (positively identified and completed examination). One body had been approved for release in 2015. The identification, examination, or release status of the remaining 48 bodies could not be determined at the time of the assessment.

It became apparent during the assessment that NCF was storing bodies unnecessarily for a lengthy period of time, contributing to the storage space shortage. A centralized data management system that could be used to track the decedent receipt date, identification status, examination completion date, and the ready-for-release date was needed. Until such a data management system can be procured, the assessors developed a spreadsheet to track each decedent and calculate the length of time each body was held at each stage of the process (e.g., length of time between identification and examination). The tool could be used to assist the mortuary operation supervisor in tracking decedents and ensuring unclaimed decedents are released for final disposition in a timely manner following the Puerto Rico statute (25 L.P.R. § 3600).

#### **Mortuary Management, Decedent Tracking, Decedent Handling**



### A107 Challenges and Lessons Learned From Having an External Expert Step Into the DNA Laboratory

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**Learning Overview:** The goals of this presentation are to: (1) promote the understanding of the challenges faced when hosting an external expert; (2) provide and understand the challenges of the external expert; (3) understand the opportunity of providing outside expertise; and (4) realize the program goals through collaboration.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating both the challenges and rewards discovered through having external experts visit the Puerto Rico Crime laboratory, speak with staff, directly observe lab conditions and resources, and work directly with staff to provide constructive recommendations. Through continued contact, relationship, and trust building, understanding of strengths and opportunities makes the best use of lab and expert time. Outside expertise liaisons provide an objective reservoir of capability, which can be leveraged to break logjams and obtain necessary resources.

On September 19, 2017, Hurricane Maria, a Category 5 hurricane, struck Puerto Rico, devastating the island population, crippling infrastructure, and leaving the vast majority of the island without power for weeks to months. The precarious fiscal situation of the island had already caused the Puerto Rico Crime Lab (Bureau of Forensic Sciences (FSB)) to lose a great number of valuable and highly trained employees, including crime scene investigators, questioned documents examiners, chemists, firearms and tool marks specialists, and digital evidence and DNA analysts. With this economic and environmental crisis at hand, the Department of Justice of Puerto Rico reached out to the American Society of Crime Laboratory Directors (ASCLD) for assistance in rebuilding its forensic capability. ASCLD responded by establishing a Board Meeting on the island to include planning sessions with Puerto Rican Crime Lab authorities to establish a consolidated response, including providing an extensive audit of operations, provision of recommendations, and application for grant funding to outsource backlogged cases and purchase new equipment.

A team of volunteer experts, including those with DNA, quality management, and laboratory operations expertise responded. After a few visits to Puerto Rico by ASCLD and the National Association of Attorneys General (NAAG) members, a Memorandum Of Understanding (MOU) agreement was established. The main goal of this MOU was to implement a laboratory assessment toward identifying the main needs. A team of four individuals visited the Bureau to meet with staff, directly evaluated laboratory infrastructure, equipment, and facilities, as well as laboratory policies, procedures, and metrics. Based on their findings, constructive recommendations were provided by the ASCLD and NAAG team.

Establishing external expert liaisons may represent a challenge. Experts must be welcomed by the host lab, with trust developed through ongoing contact. Through a trusted relationship, a better understanding of the current situation has been gained, along with what areas of the laboratory may need assistance, which can be built upon, and what solutions are a best fit to overcoming challenges of backlogs and resource issues. Recommendations must be tailored to the host lab to ensure they are receptive, that solutions will be embraced, implemented, and provide the assistance they are targeted toward.

Findings and recommendations were discussed with the Puerto Rico staff to ensure such recommendations were fit for implementation. Recently obtained equipment in the Puerto Rico FSB was in the process of validation, and additional recommendations were provided to complete needed validations, as well as to shift staff to the most beneficial components of operations. This was facilitated by a proposal for use of Rapid DNA technology in the morgue unit, freeing up necessary DNA analytical capability. A backlog reduction grant was written with support from external expertise using information provided by Puerto Rican staff. This effort not only enabled a shorter turnaround for the grant application, but also allowed Puerto Rico to apply for more grants by dividing up the work between the external support and the Puerto Rico staff.

The agreement and collaborations between the Puerto Rico FSB, ASCLD, and NAAG are a clear example of how external experts' liaisons with a host lab can provide a valuable viewpoint, adding legitimacy and voice to lab staff. The input and feedback provided have clearly aided the Puerto Rico FSB to identify their areas of need as well as to identify the most appropriate strategies to overcome challenges. In summary, the ASCLD-NAAG Puerto Rican initiative was as rewarding for the external experts as it was for the host lab: labs helping labs with shared expertise.

#### Outside Expert, DNA Lab, Disaster Recovery



### **A108 The American Board of Forensic Anthropology (ABFA) Response to the Puerto Rico Bureau of Forensic Sciences (BFS): Partnering With the National Association of Attorney’s General and the American Society of Crime Laboratory Directors**

*Dana Austin, PhD\*, Tarrant County Medical Examiner’s Office, Fort Worth, TX 76104-4919; MariaTeresa A. Tersigni-Tarrant, PhD\*, Saint Louis University School of Medicine, Saint Louis, MO 63104*

**Learning Overview:** After attending this presentation, attendees will be aware of successful means to organize and implement a forensic anthropology response to assist a medical examiner’s office in the analysis of multiple skeletal cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the organization and performance in a forensic anthropology response to an interagency medicolegal project. Lessons learned from a team of ABFA Diplomate forensic anthropologists assisting death investigation in Puerto Rico will be discussed.

This presentation details the processes of pre-deployment, deployment, and post-deployment as two groups of forensic anthropologists assisted the Puerto Rico BFS with a forensic anthropology case backlog in January and April of 2019. This backlog was, in part, due to the severe illness and resignation of Dr. Edwin Crespo Torres in 2018.

In this situation, where the scope of practice and type of assessment were unknown, the board-certified forensic anthropologists spent considerable time during the pre-deployment period planning for various contingencies and scenarios. The team was provided with an estimated number of skeletal cases requiring analysis; however, the condition of the remains and the laboratory facilities were unknown. The team created a bench notes template that encompassed the wide scope of forensic anthropological analysis: chain of custody, skeletal inventory, biological profile, trauma, postmortem interval, and taphonomy. The template was flexible to allow individual practitioners the freedom to complete analyses using the methods they found appropriate. During this pre-deployment process, a single individual served as the primary point of contact with the team-at-large and as leader of the anthropology team to facilitate communication, completeness, and a cohesive group.

During the first deployment, triage at the Puerto Rico BFS determined priority of casework and division of labor for the team. The six senior anthropologists and two graduate students needed to understand and follow the same processes of documentation with common bench notes, photography, radiography, DNA sampling, and chain of custody for transfer of the cases to the Puerto Rico BFS staff. A single anthropologist focused on ensuring Quality Assurance(QA) and Quality Control (QC) during the implementation and completion of these processes was critical. The QA/QC leader allowed for the rest of the team to concentrate on completing 47 cases, including 17 forensically significant cases, 22 cemetery remains that were not of forensic significance, 7 non-human bone cases, 2 cases comprised of “Other,” and review 14 skeletal case files to assess future needs.

Once the first deployment was completed, summaries and complete case files were provided to the Chief Medical Examiner. An inventory of available equipment, disposable supplies, and reference material was completed for anticipated future anthropology team deployment. Each anthropologist departed with their own bench notes and were provided case report templates and deadlines to complete signed case reports. Case report templates ensured that all reports were uniform in terms of the information that was provided to the Puerto Rico BFS. Once completed, the entire case file and report were reviewed by a single peer reviewer. Peer review provided another QA/QC check to ensure that the notes and case reports were complete, consistent with the template, and that the findings were sound and based on good science.

A second deployment in April 2019 included the same team leader and three new anthropologists. The Puerto Rico BFS staff was prepared for the second team, now comfortable with the processes put in place and enthusiastic because of the amount of work that had been completed on the previous deployment. The mortuary staff pulled numerous skeletal cases and staged them for the anthropologists before the team arrived. The staff also ensured workspace was available throughout the response. This allowed the second team to work more efficiently to complete even more casework, including 34 forensic cases, 17 cemetery remains, 7 non-human bone cases, and 2 cases involving other types of evidence.

This presentation provides knowledge gained during a multidisciplinary team response to a forensic laboratory facing numerous obstacles. The presentation focuses on the anthropology response, showcasing pre-deployment planning, process development, and the flexibility required to successfully perform forensic anthropological analysis in a remote, unknown medicolegal settings. The lessons learned from the response to BFS are applicable to other medical examiner offices facing similar obstacles.

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#### **Forensic Anthropology, Medicolegal Investigation, Multidisciplinary Collaboration**

## A109 Assessing Isotope Data Comparability: An Example From the Application of Isotope Testing to Unidentified Human Remains From Past Conflicts

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**Learning Overview:** After attending this presentation, attendees will have learned a straightforward method for determining a real interpretative difference that should be used to compare isotope data generated by different laboratories.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an example of assessing comparability between isotope datasets from different laboratories; the recommended practice can be easily implemented by research groups collecting and using isotope data to provenance unknown individuals.

Isotope testing has proven to be a useful tool for provenancing unidentified human remains. However, application of the technique requires reference datasets of potential source origins or populations that are sometimes compiled from remains prepared and analyzed at different laboratories. It is critical that scientists assess comparability between datasets, especially when drawing conclusions about an unknown decedent.

Inferences about similarities between a sample and available reference data are questionable when variations in isotope measurement results are not well-defined. To address this, Pestle et al. designed an inter-laboratory comparison to quantify variability in the isotopic analysis of ancient human remains; they found that approximately half the variation was due to differences in sample preparation methods, while the other half was due to differences in analytical techniques.<sup>1</sup> This led to the development of a Minimum Meaningful Difference (MMD) metric. The MMD was calculated by taking the mean pairwise inter-laboratory difference and adding four times the mean of the standard deviation of results from each participating laboratory; differences between laboratories were considered real if they exceed the MMD threshold. While the MMD represents a useful metric for assessing isotope data comparability, it also represents a worst-case scenario due to the number of participants in the inter-laboratory comparison.

A more straightforward method for assessing isotope data comparability focuses on paired samples, whereby a set of samples is prepared and/or analyzed twice to investigate practical differences between two laboratories by calculating a Real Interpretative Difference (RID).<sup>2</sup> To demonstrate this, 30 samples were collected from long bones and split for preparation as collagen and bioapatite at the Defense POW/MIA Accounting Agency (DPAA) Laboratory in Hawaii and California State University (CSU), Chico. Extracts of collagen from both laboratories were submitted to the University of California, Davis for measurement of carbon and nitrogen isotope values, while the bioapatite extracts were submitted to IsoForensics, Inc. for measurement of carbon and oxygen isotope values. Based on collagen yields, nine samples were affected by diagenesis and were not used in subsequent statistical evaluations.

Mean differences in the collagen prepared at the DPAA and CSU, Chico were 0.10‰ for  $\delta^{13}\text{C}$  values and 0.19‰ for  $\delta^{15}\text{N}$  values; while small, these differences were significant when compared to a theoretical mean of 0 (one-sample *t*-test;  $p < 0.001$  for both). There was no significant difference in the  $\delta^{13}\text{C}$  values of the bioapatite extracted at the DPAA and CSU, Chico. In contrast, there was a significant difference in bioapatite  $\delta^{18}\text{O}$  values ( $p < 0.0001$ ), with pairwise differences as large as 1.3‰. RID thresholds were calculated by adding three times the Standard Deviation (SD) of the mean pairwise differences (representing ~99% confidence) to the long-term SD of check standards used during sample analysis. Check standards included bovine liver (SD=0.07 and 0.08 for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively) and powdered marble (SD=0.09 and 0.16 for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values, respectively). Final RID values were 0.40‰ for collagen  $\delta^{13}\text{C}$  values, 0.74‰ for collagen  $\delta^{15}\text{N}$  values, 1.42‰ for bioapatite  $\delta^{13}\text{C}$  values, and 1.61‰ for bioapatite  $\delta^{18}\text{O}$  values. No pairwise difference exceeded RID thresholds for either collagen  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values. Likewise, no pairwise difference exceeded the bioapatite RID values for either carbon or oxygen isotope values.

This study demonstrates a method for assessing comparability between isotope datasets, focusing on modern human bone collagen and bone bioapatite. Results show that collagen prepared by the DPAA today can be reliably compared to previously published datasets of collagen prepared by CSU, Chico.<sup>3</sup> Comparison of bone bioapatite prepared at the two laboratories suggests that carbon isotope values are reliable, but the location of preparation has a significant impact on oxygen isotope values. More work is needed to identify and potentially control for this variable as it could have a serious impact on provenance predictions based on the measured  $\delta^{18}\text{O}$  value.

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2. Chesson, L.A., Kenyhercz M.W., Regan L.A., and Berg G.E. (2019) Addressing Data Comparability in the Creation of Combined Data Sets of Bioapatite Carbon and Oxygen Isotopic Compositions. *Archaeometry*. doi:10.1111/arcm.12480.
3. Bartelink, E.J., Berg G.E., Beasley M.M., and Chesson L.A. (2014) Application of Stable Isotope Forensics for Predicting Region of Origin of Human Remains From Past Wars and Conflicts. *Annals of Anthropological Practice* doi:10.1111/napa.12047.

### Bone Apatite, Bone Collagen, Quality Control (QC)





## A110 Using Stable Nitrogen Isotope Ratios From Fly Larvae for Postmortem Interval (PMI) Estimation

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**Learning Overview:** After attending this presentation, attendees will understand how changes in stable nitrogen isotope ratios ( $\delta^{15}\text{N}$ ) of some species of flies collected from decomposing human bodies may aid in the estimation of the PMI.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a novel application of stable isotope analysis of  $\delta^{15}\text{N}$  values of fly larvae residing on decomposing human bodies to use as an alternative to traditional PMI estimation techniques. Furthermore, this study builds on previous research on the application of  $\delta^{15}\text{N}$  ratios of decomposing muscle tissue for PMI estimation. By combining muscle and fly larvae data, seasonal differences in PMI can be estimated from the point of death through the entire skeletonization process as long as larvae are recovered.

One of the most difficult periods to estimate PMI is during initial skeletonization. Imagine recovering a surface scatter of a skeleton in late summer or early fall: Did the skeletonization process happen quickly during a hot humid summer or had a long slow winter decomposition occurred over many months? To test if PMI estimation can be improved during initial skeletonization, the putrefaction process of  $\delta^{15}\text{N}$  ratios in maggots may help. What is happening to nitrogen during decomposition and subsequent consumption by fly larvae? Nitrogen has two stable isotopes with different masses, a heavy ( $^{15}\text{N}$ ) and a light ( $^{14}\text{N}$ ) isotope. In a chemical reaction, the isotopes with different masses will react at different rates, resulting in a preferential selection of one isotope ( $^{14}\text{N}$ ) compared to the other ( $^{15}\text{N}$ ). During decomposition (e.g., putrefaction), the breakdown of amino acids produces a variety of volatile compounds, which preferentially incorporate the light isotope ( $^{14}\text{N}$ ) because it will react faster in a chemical reaction, resulting in an enrichment of the heavy isotope ( $^{15}\text{N}$ ) in the remaining decomposition fluid, which is nutrient rich for fly larvae. The impact to  $\delta^{15}\text{N}$  values as putrefaction progresses would be higher values through time recorded by fly larvae feeding on the nutrient-rich source.

This research was conducted at the Anthropology Research Facility in Knoxville, TN, an outdoor laboratory for the study of human decomposition. A total of 20 donors were enrolled in the study, placed on the surface in two trials with 10 donors each in January (winter) and July (summer) to test differential seasonality effects to the method as a PMI estimator. Blow fly larvae, an early decomposition insect, were present from February through August. Soldier fly larvae, a late stage decomposition insect, were present from June through December. Regardless of whether raw  $\delta^{15}\text{N}$  ratios or  $\Delta^{15}\text{N}$  ( $\Delta^{15}\text{N} = \delta^{15}\text{N}_{\text{Day X larvae}} - \delta^{15}\text{N}_{\text{Day 1 muscle}}$ , with Day 1 as the initial fresh tissue sample to normalize the baseline of different donors), the blow fly larvae did not predict seasonal differences for PMI estimation ( $p=0.61$ ), while soldier fly larvae could be used to predict winter vs. summer placement ( $p=0.00$ ).

The winter trial ( $n=43$ ) had soldier fly larvae arrive after five months of decomposition with a mean  $\delta^{15}\text{N}$  of 27.4‰ (ISD=6.4) and range of 18.0 to 43.2‰. The summer trial ( $n=66$ ) had soldier fly larvae arrive after three weeks of decomposition with a mean  $\delta^{15}\text{N}$  of 18.0‰ (ISD=4.0) and range of 10.7 to 30.0‰; however, there are only four samples above 23.3‰. With a sectioning point of 21.7‰ calculated from the mean  $\delta^{15}\text{N}$  of the soldier fly larvae, the overall accuracy rate for the sample was 79.8%. For conservative PMI estimates, it is recommended that  $\delta^{15}\text{N}$  values from soldier fly larvae be used as general indicators of winter versus summer placement when values are above 24‰ vs. below 19‰, respectively.

This East Tennessee pilot study indicates that the significant influence to  $\delta^{15}\text{N}$  values of soldier fly larvae is time of decomposition prior to arrival of this late stage decomposition insect. The impact to  $\delta^{15}\text{N}$  ratios as putrefaction progresses would be higher values later in decomposition recorded by soldier fly larvae. This initial study indicates there is value in the method as a PMI estimate for recently deceased individuals when soldier fly larvae are present. This relationship does not hold for early stage insect activity, as seen in  $\delta^{15}\text{N}$  values of blow fly larvae.

### Stable Nitrogen Isotope Ratios, Postmortem Interval Estimation, Fly Larvae

## A111 Crossing Borders: International and Multidisciplinary Applications of Isotope Analysis in Forensic Anthropology

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**Learning Overview:** The goal of this presentation is for attendees to learn about an integrated tool using isotope data mixed with socio-economic status to be applied forensically for provenance region, in the medicolegal context, but also to add information about unknown persons.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new proxy for tracking human movements under the worldwide “global supermarket” dietary trend, potentially adding more detailed connections to narrow predictions of a region of origin and in the identification of unknown persons.

Several works have used the multi-isotope approach to track the geographical origin of human remains as well as human movements in forensic anthropology, but only a few studies have used it in association with some socioeconomic status and dietary behavior changes. These studies have demonstrated the progressive substitution of local staples for industrialized processed foods in the developing regions of the world (the so-called nutrition transition process), increasingly recorded in respective rural areas. In this context, a new proxy for tracking human movements under the worldwide “global supermarket” dietary trend was explored.

The spatial distribution of  $\delta^{13}\text{C}$  in contemporary human fingernails, representing the food sources and dietary practices in a region, was associated with the globally well-recognized Human Development Index (HDI) from the United Nations Organization. The  $\delta^{13}\text{C}$  of fingernails from approximately 4,000 Brazilian inhabitants living in rural and urban areas spatially distributed in the territory was analyzed. The HDI at the municipality level was associated with the average  $\delta^{13}\text{C}$  of fingernails for the corresponding municipality. A sub-dataset of approximately 2,000 samples of fingernails from pairwise cities and isolated villages in two different regions of Brazil, the Amazon tropical forest and the Central Brazilian savannas, were analyzed to explore the possibility of using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of fingernails to differentiate between urban and village areas and also between males and females living at the same locality.

At the country level, there was a positive spatial correlation between the  $\delta^{13}\text{C}$  and HDI (0.67;  $p < 0.01$ ). The highest  $\delta^{13}\text{C}$  and HDI were found in the Southeast and South Brazilian regions, followed by the Center-West region. The increase in the number of food items derived from  $\text{C}_4$  plant resources and the replacement of food items derived from  $\text{C}_3$  plant resources was associated with higher ultra-processed food purchases as a proportion of overall household food purchases. On the other hand, the  $\delta^{13}\text{C}$  from the North and Northeastern regions had the strongest link to  $\text{C}_3$  food sources, especially in their rural areas. At the regional urban-rural gradient, fingernails from individuals living in agricultural regions were coupled to local carbon and nitrogen isotope composition, while fingernails from individuals living in more urban areas had the most decoupled isotopes signatures from the local ecosystems. However, cases in which the villages were more connected to the urban areas showed a more similar diet to the residents of the urban areas. Also, the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of inhabitants of villages with high consumer buying power tended to be more decoupled from the local landscape. At the local level, males from the isolated villages had higher  $\delta^{15}\text{N}$  and lower  $\delta^{13}\text{C}$  than females, while in the cities, there was no difference between them, indicating that males are still taking more protein from freshwater fish and bushmeat, indicated by a strong  $\text{C}_3$  signal and high  $\delta^{15}\text{N}$  compared to processed meat. Such differences among country, regional, and local levels resulted in a significant variation in fingernails’ stable isotope ratios according to the information related to both regional food sources and dietary practices but also to the degree of market integration of each region.

This integrated approach proved to be useful as a new tool to be used in human nutrition studies, especially in tracking human movements in forensic anthropology studies in the contemporary world. This integrated tool could then be applied forensically for the provenance region, adding information about missing persons.

### Socio-Economic Status, Isotopes in Fingernails, Human Movements

## A112 Isotopic Analyses of New York City's Unidentified Individuals: Differentiating Migrants From Local Residents in a Multicultural Metropolitan Context

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**Learning Overview:** After attending this presentation, attendees will understand how geolocation and dietary isotopic data are used to aid in differentiating migrants from local residents from a sample of long-term unknown individuals in New York City, a large, urban multicultural region with high residential mobility.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving understanding of the complexities of employing geolocation and dietary isotopic methods for decedent identification purposes in a multicultural metropolitan context.

Isotopic analyses of human remains provide geolocation and dietary information to the biological profile, furthering efforts to identify long-term unknown individuals. Geolocation isotopes including oxygen ( $\delta^{18}\text{O}$ ) and strontium ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) and dietary isotopes such as carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) are commonly employed to indicate geographic origins and residential mobility of unidentified decedents within the United States and globally.<sup>1-3</sup> Since an individual's isotopic values are derived from ingested food and beverages during life, the homogenization of foods via the "supermarket effect" has potential for blurring regional distinctions for geolocation purposes. Moreover, culturally influenced food traditions pose interpretative complications, especially in multicultural metropolitan regions such as New York City.

In this study, multiple isotopic systems were employed to analyze tissue sets (hair, nail, bone, enamel) of 28 forensic cases of unknown identities in the custody of the New York City Office of Chief Medical Examiner (NYC OCME) to characterize dietary practices and residential histories. Bone and enamel apatite  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  analyses of the 28 individuals and hair  $\delta^{18}\text{O}$  analyses of a subset of 8 individuals were acquired from IsoForensics, Inc. Additional bulk bone collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and incremental enamel  $^{87}\text{Sr}/^{86}\text{Sr}$  values via laser ablation were contributed to the case profiles. Hair  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of 42 living residents of New York City were analyzed and used as a dietary comparative dataset.  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  values of unidentified remains were compared to published isoscapes based on individuals of known residence and tap water values using established conversion equations.<sup>2,4,5</sup>  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were incorporated into dietary models of protein consumption and general dietary groups.<sup>6</sup>

Of the 28 NYC OCME forensic cases, enamel analyses yielded average imbibed water  $\delta^{18}\text{O}$  values  $-7.6\text{‰}$ , ranging from  $-12.9\text{‰}$  to  $2.0\text{‰}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$  values of 0.7128, ranging from 0.7067 to 0.7258. Based on enamel  $\delta^{18}\text{O}$  values alone, 14 individuals resided locally during childhood years. According to combined enamel  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  data, only 3 individuals were interpreted as local to NYC during childhood years. Bone apatite  $\delta^{18}\text{O}$  values suggest 19 individuals resided locally during adulthood. From the 8 hair and nail  $\delta^{18}\text{O}$  values, 2 individuals were identified as local residents within months of death. Bone collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values averaged  $-15.9\text{‰}$  and  $10.1\text{‰}$ , respectively, and indicate a range of diets ( $\delta^{13}\text{C}=-19.0$  to  $-12.9\text{‰}$ ;  $\delta^{15}\text{N}=7.3$  to  $11.7\text{‰}$ ). Modern NYC residents have lower  $\delta^{13}\text{C}$  values than the case sample, indicating relatively more  $\text{C}_3$ -based diets. Six individuals have  $\delta^{13}\text{C}$  values higher than  $-13\text{‰}$  and lower than  $-17\text{‰}$ , which is suggestive of non-United States and/or culturally influenced dietary practices.<sup>3</sup>

Two cases are explored in depth, both of which are interpreted to have migrated into the United States. Multiple isotopic data of one individual with an identity confirmed through DNA illustrate overlapping United States and non-United States isoscapes and a  $\text{C}_3$ -based dietary practice, which combined led to a misidentification of the individual's potential residences. Data from a second case, still pending identification, yielded a heavily  $\text{C}_4$ -based dietary practice and an extremely wide range of  $^{87}\text{Sr}/^{86}\text{Sr}$  values visible through laser ablation, which strongly suggest a non-United States residence. These two cases are used to demonstrate methodological limitations and areas of potential for determining whether an individual crossed a border in his/her lifetime.

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### Isotopic Analysis, Unidentified/Unknown, Forensic Anthropology



## A113 Isotope Analysis in Modern Colombian Teeth: A Forensic Application

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**Learning Overview:** After attending this presentation, attendees will have learned the results of isotopic research in Colombia, which aims to evaluate the usefulness of a multi-isotope approach using carbon ( $\delta^{13}\text{C}$ ), oxygen ( $\delta^{18}\text{O}$ ), strontium ( $^{87}\text{Sr}/^{86}\text{Sr}$ ), and lead ( $^{206}\text{Pb}/^{207}\text{Pb}$ ,  $^{208}\text{Pb}/^{204}\text{Pb}$ ) isotopes for the prediction of region-of-origin of skeletal remains in Colombia.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by revealing: (1) if Colombian isotopic data differs within the country and between those of other countries; (2) which isotope system can be most useful to determine if an individual is likely to be of Colombian origin; (3) if regional dietary patterns were observed; and (4) if  $\delta^{18}\text{O}$  in enamel bioapatite varies according to altitude as expected.

The application of stable isotopes to human identification is a rapidly expanding field in forensic science due to the valuable information isotopes can impart about the geographic origin and geographic life history of an individual.

Currently, the country of Colombia faces a massive identification crisis because of long-term violence due to socio-political conflict and criminal activity. The government databases estimate that there are currently between 10,000 and 22,000 unidentified persons; therefore, methods that can assist in narrowing identification possibilities are needed.<sup>1</sup>

The sample was composed of modern permanent teeth from Colombia and published Colombian data. The sample included 97 donated teeth of individuals with known demographic information from the cities of Bogota, Cali, and Neiva (approved by the Institutional Review Board of Binghamton University), as well as published data from 61 teeth from the Medellin skeletal collection from individuals with known demographic information.

Overall, carbon isotopes in the tooth enamel carbonate show a diverse range with  $\delta^{13}\text{C}$  values between -14.09‰ to -2.80‰, which falls in the range of reported values from the United States. However, there are significant differences in  $\delta^{13}\text{C}$  values between some Colombian cities (e.g., Cali, Medellin, and Cartagena). Oxygen isotopes in the tooth enamel carbonate show a range of  $\delta^{18}\text{O}$  values between -9.64‰ to -2.74‰; a slight trend in decreasing  $\delta^{18}\text{O}$  values with increasing altitude is observed, in addition to differences between individuals who live in the coast and highlands (e.g., Cartagena vs. Bogota).

Colombian tooth enamel has  $^{87}\text{Sr}/^{86}\text{Sr}$  values between 0.70475 and 0.71839, which fall within the range of reported values from other regions (e.g., Mexico). However, differences between cities are observable (e.g., Cali vs. Bogota). Analysis of the lead isotopic composition is  $^{206}\text{Pb}/^{204}\text{Pb}=17.972$  to  $18.976$ ,  $^{207}\text{Pb}/^{204}\text{Pb}=15.576$  to  $15.683$ , and  $^{208}\text{Pb}/^{204}\text{Pb}=37.819$  to  $38.602$ . Lead isotopic data seems homogeneous; however, slight differences between Colombia, United States, and European values are observed.

This presentation will provide insights about isotope analysis in Colombia and the utility of a multi-isotope approach for determining place-of-origin of an individual. In addition, by generating a Colombian baseline for future place-of-origin predictions, it contributes to the identification and search for missing people in Colombia. The differences found between countries will be useful for forensic purposes on a continental and global scale. This Colombian data will have international applications, such as helping with identifying missing people who die crossing the border or by natural or human-made disasters.

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### Isotope Analysis, Colombia, Forensic Anthropology

### A114 Examining Stable Isotope Ratios From Victims of the Internal Armed Conflict in Peru (1980s–2000) to Establish Local or Non-Local Geographic Origins: A Preliminary Analysis of the Victims of the Military Base Los Cabitos, Ayacucho, Peru

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**Learning Overview:** The objective of this presentation is to educate attendees about using stable oxygen carbon and nitrogen isotope analyses as a method for evaluating whether a deceased individual is from the geographic region where a body was disposed. This presentation will also provide attendees with specific information about a case from the Internal Conflict in Peru that left hundreds of unidentified bodies at the Los Cabitos exhumation site in Ayacucho, Peru, the geographical heart of the conflict between the Shining Path and the Peruvian State.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting methods and results that will teach attendees about the benefits and limitations of using stable isotope analysis and missing persons' databases as part of the methodological toolkit for moving closer to identifying unknown persons.

This study applied isotopic analyses to evaluate whether unidentified persons recovered from the Los Cabitos exhumation site in Ayacucho, Peru, were from the local Ayacucho region. Those bodies were disposed there during the Time of Violence in Peru in the 1980s–2000s. This is the first phase of isotopic analysis of the victims' bodies, which will be used to estimate possible geographic origins of the unidentified persons. The oxygen isotope ratios ( $^{18}\text{O}:^{16}\text{O}$ ) in drinking water sources are affected by latitude, temperature, elevation, distance from the coast, and other factors. If a person consumes local water, the oxygen isotope ratio in human tissues should align with local water drinking sources. Carbon isotope ratios ( $^{13}\text{C}:^{12}\text{C}$ ) in humans are determined by the plants consumed (and of the animals that eat those plants), and nitrogen isotope ratios ( $^{15}\text{N}:^{14}\text{N}$ ) from collagen vary depending on protein sources, among other factors (e.g., fertilization of crops). Depending on cultural/ecological contexts, certain ranges of carbon and nitrogen isotope values are expected for particular populations (e.g., a high-altitude, subsistence-based pastoralist from the 1980s in the Andes is likely to have lower carbon isotope ratios due to less access to maize, which grows in lower altitudes, and lower nitrogen isotope ratios because of little-to-no consumption of marine resources).

**Research Goals:** This presentation documents carbon and oxygen isotope ratios from dental carbonates and nitrogen isotope ratios from bone collagen from 45 unidentified persons from the Los Cabitos exhumation site. If outliers are identified based on isotope data, then that likely suggests that those individuals are not from the local Ayacucho region. Those "outlier" individuals can then be compared to the missing persons database of individuals who were imprisoned at Los Cabitos in the 1980s and 1990s.

**Methods:** Two enamel powder samples were taken from each tooth. Chemical preparation of the specimens was conducted at the Vanderbilt Bioarchaeology and Stable Isotope Research Laboratory (BSIRL) and processed at the Yale Analytical and Stable Isotope Center on a Thermo DeltaPlus Advantage (for carbon and nitrogen in collagen) and a Thermo<sup>TM</sup> DeltaPlus<sup>TM</sup> XP (for carbon and oxygen in dental carbonates). Carbon and oxygen isotopes are reported relative to the Vienna Pee Dee Belemnite (VPDB) standard:  $\delta^{13}\text{C} = \left( \left[ \frac{^{13}\text{C}/(^{12}\text{C}_{\text{sample}})}{^{13}\text{C}/(^{12}\text{C}_{\text{standard}})} - 1 \right] \times 1,000 \right)$  and  $\delta^{18}\text{O} = \left( \left[ \frac{^{18}\text{O}/(^{16}\text{O}_{\text{sample}})}{^{18}\text{O}/(^{16}\text{O}_{\text{standard}})} - 1 \right] \times 1,000 \right)$ . Nitrogen isotope ratios are reported relative to Atmospheric Nitrogen (AIR):  $\left( \left[ \frac{^{15}\text{N}/(^{14}\text{N}_{\text{sample}})}{^{15}\text{N}/(^{14}\text{N}_{\text{standard}})} - 1 \right] \times 1,000 \right)$ .

**Results:** Results showed that among the enamel carbonate samples, the mean  $\delta^{18}\text{O}_{\text{PDB}} = -9.0\text{‰}$  (s.d.=1.1) and the mean  $\delta^{13}\text{C}_{\text{PDB}} = -8.2\text{‰}$  (s.d.=1.9);  $N=89$  enamel samples for both isotopes. From the bone collagen samples, the mean  $\delta^{13}\text{C}_{\text{PDB}} = -16.2\text{‰}$  (s.d.=1.9) and the  $\delta^{15}\text{N} = 9.8\text{‰}$  (s.d.=0.5);  $N=12$  bone collagen samples for both isotopes. There are two outliers with both oxygen and carbon isotope values that are more than four times the standard deviation for one individual and four times the standard deviation for oxygen for the other. These isotope data strongly suggest that these two individuals spent their childhood in a distant locale, outside of the Ayacucho region. The bone collagen data suggest that the individuals then moved to Ayacucho, residing there for at least the last five to ten years before death, consuming diets similar to all of the other natal individuals. There are 16 individuals who were born outside of the Department of Ayacucho in the missing persons database for the Los Cabitos military complex.

**Conclusion:** These results can be used to evaluate whether the outliers were one of the non-local people in the missing persons database. Although isotope studies as a sole method do not arrive at positive identifications, these methods do narrow the range of options, which may aid in more focused DNA studies and other identification techniques.

#### Stable Isotopes, Peru, Shining Path Conflict



## A115 Spatial Distributions of Isotope Ratios in Tap Water, Hair, and Teeth From Latin America for Region of Origin Predictions of Unidentified Border Crossers

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**Learning Overview:** The goal of this presentation is to introduce recent applications of stable isotopes and “isoscapescapes” for predicting region of origin of unidentified remains cases of Undocumented Border Crossers (UBCs) from the United States-Mexico border. This research provides an overview of the process and implications of building an isoscape from tap water and hair samples and discusses how these reference data provide a framework to predict region-of-origin of deceased migrants from Arizona and South Texas.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the use of reference baseline isotope data to refine predictions of region of origin of unidentified remains of UBCs and to highlight both the limitations and challenges of these approaches.

Each year, hundreds of UBCs are found deceased along the United States-Mexico border, with the highest volume of deaths occurring in Arizona and South Texas. UBCs are among the most difficult individuals to identify due to the lack of antemortem identification records and challenges in obtaining family reference DNA samples. Stable isotope analysis of human tissues represent another line of evidence to provide investigative leads for identification efforts of UBCs from Arizona and South Texas. Previous research on carbon isotopes identified significant dietary differences between the continental United States and Latin America, providing a useful screening tool. The addition of oxygen and strontium isotopes provides a more precise geospatial tool for narrowing region of origin since these isotopes reflect the sources of drinking water and food, respectively. To date, only crude isoscape maps exist for most regions within Latin America due to the lack of baseline reference data, which hinders efforts for predicting region of origin. This presentation adds to the growing baseline data for Latin America by presenting data on regional variation of isotopes in water and hair.

Tap water ( $n=158$ ) and hair ( $n=101$ ) samples were collected from 51 towns and 32 cities throughout Mexico to serve as a reference database for assigning deceased unidentified UBCs to potential regions of origin. Tap water samples were measured for oxygen and hydrogen isotopes, and hair samples were measured for oxygen isotopes. Water ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ ) and hair ( $\delta^{18}\text{O}$ ) isoscapes were generated using ArcGIS® based on the spatial distribution of the reference sample using Moran’s  $I$  to quantify spatial autocorrelation (Spatial Statistics Toolbox, ArcGIS® 10.6). The tap water ratio data were defined by Moran’s indices of 0.843 and 0.856 ( $z$ -scores of 7.5 and 7.6 and  $p < 0.000001$  for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ , respectively).

Carbon and oxygen isotopes were also measured in tooth enamel bioapatite from deceased UBCs from Pima County, AZ, ( $n=30$ ) and Brooks County, TX ( $n=41$ ). Permission to sample these remains was granted by the Pima County Office of Medical Examiner and Texas State University for the Arizona and Texas samples, respectively. Various lines of evidence indicate that the majority of UBCs found in Arizona derive from Mexico, whereas the majority of UBCs found in South Texas derive from Central America (primarily Guatemala, Honduras, and El Salvador). The mean enamel bioapatite  $\delta^{13}\text{C}$  value is  $-6.4\text{‰}$  ( $\pm 2.3\text{‰}$ , 1 SD; range= $10.2\text{‰}$ ) for the Arizona samples versus  $-5.4\text{‰}$  ( $\pm 3.0\text{‰}$ , 1 SD; range= $13.0\text{‰}$ ) for the South Texas samples, demonstrating a non-significant difference ( $t=1.594$ ,  $df=69$ ,  $p=0.115$ ). The mean enamel bioapatite  $\delta^{18}\text{O}$  value is  $-4.4\text{‰}$  ( $\pm 3.2\text{‰}$ , 1 SD; range= $0.9\text{‰}$ ) for the Arizona samples versus  $-5.5\text{‰}$  ( $\pm 1.2\text{‰}$ , 1 SD; range= $7.2\text{‰}$ ) for the Texas samples, a statistically significant difference ( $t=-3.949$ ,  $df=68$ ,  $p<0.001$ ). However, the differences in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  between UBCs from Arizona and South Texas are small, and their source populations within Mexico and Central America cannot be determined using the isotope data currently available.

Three case studies from Arizona are used to highlight the application and limitations of the tap water and hair oxygen isotope isoscape based on individuals of known origin (i.e., UBCs who have recently been identified). The isoscape predictions for these cases are consistent with the known origin of these individuals, but include several possibilities within Mexico. The addition of strontium isotope baseline data will aid in refining these predictions in the future.

The ultimate goal is to incorporate the data into a larger network of tap water and human hair isoscapes in order to establish probability densities for the most probable regions of origins for the deceased undocumented migrants.

### Isotope Analysis, Undocumented Border Crossers, Forensic Anthropology



### A116 Considerations for Isotope Analysis of Human Hair: The Impact of Postmortem Environmental Exposure

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**Learning Overview:** After attending this presentation, attendees will appreciate the impact of outdoor exposure upon isotope ratios of human hair over time.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing possible concerns for isotope ratios obtained from human hair exposed to outdoor environments and by encouraging discussion of the impacts of taphonomic processes upon isotope analysis of human remains.

Isotope analysis is a tool anthropologists have adopted from other disciplines, crossing into biochemistry, ecology, and geology to better understand the relationships between people and their environments. As forensic anthropologists use isotope analysis with growing frequency in order to provide additional information about unidentified human remains, it is necessary to assess the limitations of this analysis. While isotope analysis does not provide individual positive identification, predictions about diet and geographic travel prior to death can generate new information in the absence of other leads. Because hair grows at a predictable rate and provides information about the weeks and months prior to death, it has been used in forensic contexts and is particularly useful in determining whether an individual was local to a particular area or possibly traveled from elsewhere. Previous research and predictive geospatial modeling (i.e., isoscapes) were conducted using pristine hair samples from salons, which do not reflect the conditions typical of forensic recoveries. This study addresses how isotope ratios of human hair are impacted by outdoor exposure over time.

Two outdoor laboratories were used: the Anthropology Research Facility in Knoxville, TN, and the Forensic Anthropology Research Facility in San Marcos, TX. Body donors with known residence histories ( $n=46$ ) were enrolled in the study. Hair samples that were collected prior to outdoor placement were compared to hair samples collected at various times throughout the decomposition process, with exposure times ranging from 22 days to 1,140 days (approximately 3 weeks to 3 years) postmortem. All samples were analyzed for carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ), hydrogen ( $\delta^2\text{H}$ ), and oxygen ( $\delta^{18}\text{O}$ ) isotope ratios, and a subset of 18 pairs were analyzed for strontium ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) isotope ratios. No statistically significant differences were observed between pre- and post-exposure samples for  $\delta^{13}\text{C}$  ( $p=0.897$ ) and  $\delta^{18}\text{O}$  ( $p=0.267$ ) values. Significant differences were observed in  $\delta^{15}\text{N}$  ( $p=0.013$ ),  $\delta^2\text{H}$  ( $p<0.001$ ), and  $^{87}\text{Sr}/^{86}\text{Sr}$  ( $p<0.001$ ), though it is important to consider meaningful interpretive differences versus statistically significant differences when comparing values. Additional statistical evaluation using linear growth models revealed that number of days of exposure, location of placement (TN or TX), and placement condition (surface or burial) are variables that impact isotope ratios of human hair.

While this study revealed that postmortem isotope ratios from human hair may be impacted by outdoor exposure, the observed differences did not significantly impact the dietary and geographic travel predictions made from the measured isotope values. With the ever-growing number of unidentified remains of migrants of unknown geographic origin, isotope analysis is an essential tool for the forensic anthropologist. Isotope analysis of hair continues to show promise for providing valuable information that can assist with the identification of unknown human remains, especially as understanding of the effects of taphonomic processes upon isotope ratios continues to grow.

#### Isotope Ratios of Human Hair, Isotope Analysis, Decomposition



## A117 Differentiating Handsaw Tooth Shape Based on the Analysis of the Kerf Floor Contour

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**Learning Overview:** After attending this presentation, attendees will understand if kerf floor contour can be used to accurately predict handsaw tooth shape in dismemberment cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by further validating the work of Symes et al. on saw analyses by indicating whether or not kerf floor shape can be used to reliably predict tooth shape in rip and crosscut handsaws with varying Teeth Per Inch (TPI).<sup>1</sup>

Nearly all dismemberment cases have at least one incomplete cut (a.k.a. false start) available for analysis. The kerf floor and walls contain the most information on class characteristics, including tooth shape: rip vs. crosscut.<sup>1</sup> Rip saws, with flat chiseled teeth at a 90-degree angle, are designed to cut with the grain of wood and produce a U-shaped/concave kerf.<sup>1</sup> Crosscut saws have teeth that are filed usually at 70-degrees with every other tooth being filed on the opposite side. They are designed to cut against the grain of wood and produce a W-shaped/convex kerf.<sup>1</sup> The differences in the contour of the kerf floor are therefore correlated with the differences in tooth design.

This research was designed to answer four questions: (1) Do rip saws always produce a U-shaped kerf floor and do crosscut saws always produce a W-shaped kerf floor? (2) Can kerf floor shape *always* be used to differentiate rip vs. crosscut saws? (3) Does teeth per inch impact visibility of the kerf floor shape? and (4) Does new vs. used blade affect the ability to differentiate saw tooth shape?

Two crosscut and three rip handsaws with alternate set teeth were selected from a local hardware store for the study. Each blade was new and was used by one individual to make 30 consecutive incomplete cuts on dry non-human long bones ( $n=150$  cuts total). Cut order sequence on the bones was randomized and labeled 1–150 prior to beginning, so that the second observer analyzing kerf floor shape did not know which of the five saws or which cut number per saw was being analyzed. Each kerf floor was then blindly analyzed using a digital microscope at 30x magnification. Real-time depth-up composition was used to generate a 3D model of each kerf. Next, the profile function was used to generate a standardized profile contour of each cut along the length of the entire cut. The profile shape was then visually classified by the observer as U-shaped or W-shaped. If any portion of the kerf midline was convex, the cut was classified as a W-shape.

Of the profile contours, 98% ( $n=147/150$ ) matched the expected shape based on tooth type: rip vs. crosscut. Two crosscut saw cuts and one rip saw cut were misidentified. In the case of one of the misidentified crosscut saw cuts, review of the file revealed that depth had not been calculated at the deepest location within the cut by the 3D function of the microscope; therefore an incorrect kerf floor contour was calculated. The other two misidentified cuts were true misidentifications. In all but one case, the entirety of the cut was U-shaped or wide V-shaped for the rip saws ( $n=89$ ). Contrary to expectations, the cuts made by crosscut saws varied along the length of the cut from W-shaped to concave, with the more narrow portions of the cut tending to exhibit the flatter or concave shape. This is likely contributed to blade drift of the alternate set saw. However, at least one portion of the cut exhibited a distinct W-shape for accurate identification. Often, one side of the W was uneven, with the peak extending deeper. This was more pronounced with the lower TPI saw. Profile shape for the 30<sup>th</sup> cut for each saw was compared to the first cut made with each saw. The last cut with each blade was as visible as the first cut for all five saws and, in fact, the misidentified cuts were actually mid-sequence: cut 17/Saw A and cut 10/Saw D. Overall, kerf floor contour can be used to accurately predict handsaw tooth type, and results from this validation correspond to those found by Symes et al.<sup>1</sup> In instances where cuts exhibit both W-shaped and U-shaped sections along the length of the cut, the saw should be classified as a crosscut saw.

### Reference(s):

- <sup>1</sup> Symes, S.A., E.N. Chapman, C.W. Rainwater, L.L. Cabo, and S.M.T. Myser (2010) *Knife and Saw Toolmark Analysis in Bone: A Manual Designed for the Examination of Criminal Mutilation and Dismemberment*. Report, National Institute of Justice, Grant 2005-I-J-CX-K106.

### Trauma Analysis, Saws, Dismemberment





### A118 Forensic Fractography of Bone Using Computed Tomography (CT) Scans

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**Learning Overview:** After attending this presentations, attendees will have learned how to apply principles of bone fractography to fracture surfaces reconstructed from CT scans. Attendees will have also gained a deeper understanding of how 3D models can contribute to skeletal trauma analysis, as well as certain limitations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing additional information on a methodology that can assist in the analysis of fractures from CT scans. As the use of CT expands worldwide, medical examiners (as well as clinicians) will have additional tools for analyzing skeletal fractures using virtual 3D methods in a non-invasive manner.

Fractography is the science of fracture surface morphology and its relationship to crack propagation. This well-established and validated science can be used to assess a material's mechanical properties and determine the underlying mechanisms leading to material failure. Recent studies and case reports have demonstrated the utility of fractography in forensic anthropology for understanding the directionality of impact/force application and fracture propagation on broken bones by assessing features of the fracture surface. Specifically, the presence and orientation of fracture surface features, including bone mirror, arrest ridges, bone hackle, wake features, and cantilever curl, can be used to reliably determine the point of fracture initiation and the direction of crack propagation. However, these previous studies utilized processed bone and directly examined the fracture surface features visually and microscopically. Removal of soft tissue from complete or mostly complete remains may be impractical in some cases, and assessments may be expedited if fracture surfaces could be assessed without maceration.

This study investigated whether the science of fractography can be applied to reconstructed CT scans. As a test of the method, a series of clinical scans of individuals with traumatic lower extremity injuries from patients that presented to a Level 1 Trauma hospital for assessment were obtained under Institutional Review Board (IRB) approval. The scans were performed using a Philips Brilliance 64 slice CT scanner under a standard trauma scan protocol. Relevant images included thin slice data sets of the lower extremities, acquired using bone and soft tissue algorithms. The fracture surfaces were visualized as 3D computational models in the volume rendering software package Mimics® Innovation Suite version 22 and 3-Matics version 14 Materialise. The 3D models were then exported as 3D .pdfs for fractography assessment.

Using this approach, some fractographic surface features were apparent, including bone mirror, arrest ridges, and cantilever curl, which are indicative of crack propagation direction and therefore the direction of impact. Due to resolution limitations, the amount of fracture surface detail visible in the CT scans is notably less than using direct observation of the bone surface, but diagnostic features of crack propagation were still apparent in some cases. As noted in previous bone fractography studies, features were less often identified when cortical area was smaller, and comminuted fractures also made surface visualization and feature identification more challenging. The selection of 3D modeling software may also affect results. When fracture surface features can be visualized and evaluated on CT scans, this may eliminate the need for skeletal processing, and perhaps even be applied in clinical as well as forensic contexts.

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#### Fractography, Skeletal Trauma Analysis, Crack Propagation

## A119 The Application of Fractography in Trauma Analysis of Complex Long Bone Fractures

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**Learning Overview:** After attending this presentation, attendees will have learned about the utility of fractography in identifying point of failure and direction of crack propagation in complex long bone fractures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding to a growing body of research on the application of fractography to skeletal trauma analysis. This current study contributes new data from concentrated four-point bending experiments in which fractographic features were compared directly to high-speed video of fracture propagation.

Fractography refers to the study of fracture surface features and the relationship of these features to crack propagation.<sup>1</sup> Fractographic methods have been applied to a variety of materials to interpret the causes of failure. Recently, Christensen et al. proposed a method for applying fractography to the analysis of skeletal trauma in forensic anthropology cases.<sup>2</sup> They described and investigated several fractographic features, including bone mirror, bone hackle, arrest ridges, and cantilever curl, in a sample of human femora that failed in three-point bending experiments. Using these features, they reported low inter- and intra-observer error identifying fracture initiation and estimating fracture propagation in cortical bone.

While these results are promising, there is a need to clarify the strengths and limitations of fractography in skeletal trauma analysis through additional research. In the previous study, each specimen under three-point bending exhibited only one primary fracture. This allowed for a relatively straightforward assessment of one of two complementary fracture surfaces. However, it is of interest to assess whether fractography can help resolve fracture initiation and propagation in more complex cases of skeletal trauma.

The current study investigates the use of fractography in a sample of femora fractured under another set of controlled loading conditions: concentrated four-point bending. Unlike three-point bending, specimens fractured under concentrated four-point bending exhibited complex, “compression wedge” fracture patterns involving multiple fragments.

The goals of this study were: (1) to assess the presence/absence of fractographic features in this sample; and (2) to assess whether these features could be used to interpret fracture propagation. The materials assessed in the current study include six whole, biomechanically fresh human femora failed in concentrated four-point bending experiments as a part of a previous replication study of the work of Martens et al.<sup>3,4</sup> All experiments were filmed with a high-speed camera at 40,000 frames per second (fps) in order to capture fracture initiation and propagation.

The fracture surfaces of each bone fragment were examined macroscopically using oblique lighting and a low power microscope. The presence or absence, anatomical locations, and orientations of four fractographic features were noted: bone mirror, bone hackle, arrest ridges, and cantilever curl. These features were used to interpret the location of initial failure and direction of crack propagation. Finally, fractographic interpretations were compared to the real sequence of fracture events captured on video.

Twenty-eight bone fragments were examined across the six specimens in the study (mean=4.66 fragments per impact). At least one fractographic feature was present on each of the 28 fragments. In each specimen, bone mirror occurred only in the area corresponding to initial failure near the tension surface. As fracture initiated from exactly one location in each experiment, only fragments with a fracture surface corresponding to that location exhibited bone mirror.

Arrest ridges and/or cantilever curl were present where fractures terminated. All six specimens exhibited multiple termination sites; these features were therefore observed in more fragments than bone mirror. Bone hackle was the feature most commonly observed across fragments, although it was subtler in appearance than bone mirror, arrest ridges, and cantilever curl.

In all six specimens, the fractographically inferred direction of fracture matched to the actual fracture propagation direction observed on high-speed video. These results indicate that the fractographic features described by Christensen and colleagues are informative of initial failure and crack propagation even in cases involving complex, compression wedge fractures.<sup>2</sup>

In forensic anthropology, trauma analysis is moving beyond simple descriptions and fracture typologies. Instead, investigators are generating more nuanced interpretations of sequential failure modes, from fracture initiation to fracture termination. Fractography demonstrates promise as one tool to aid in such interpretations. For cases involving complex fractures, reconstructing crack development provides an important foundation for subsequent interpretations, such as bending direction of a long bone in blunt force trauma.

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### Trauma Analysis, Fractography, Blunt Force Trauma

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### A120 The Repository of Antemortem Injury Response (REPAIR): An Invaluable Online Resource for Known Age Fractures for Comparison and Research

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**Learning Overview:** After attending this presentation, attendees will better understand the Fracture Healing Database, how to contribute samples to the database, and how the database can be accessed and used as a comparative tool in cases involving cranial fracture repair.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a tool for comparison and interpretation of cranial fractures as they relate to time since injury and the influence of age on healing variation.

The genesis of the cranial fracture healing project began as a persistent call for understanding and interpretation of cranial injuries within the context of accidental versus non-accidental trauma. As such, the goals of the project are to simultaneously create a method for human cranial fracture histology, build a database of cases with known injury times, conduct an evaluative intra- and inter-observer study to create a histomorphologic tool for evaluating cranial fracture healing, and provide an interpretive framework for inferring time since injury of cranial fractures.

Searchable and easy-to-use internet-based databases allow forensic practitioners to use and contribute data and conduct research to address specific needs in the medicolegal community. Therefore, a secure web-based data system was created for this project using Occupational Research and Assessment (ORA), since the company has experience with data management for medicolegal purposes. Varying levels of database access are granted dependent on the need of the particular user, including administrator, submitting agency, and forensic professional. Submitting agencies can enter their case information directly into the database, including relevant photographs, radiographs, and documents. Prior to these cases becoming visible to other submitting agencies or forensic professionals, administrators check the submitted data to ensure there is no personal health information or case numbers included prior to publishing.

The database is organized by case, with the accompanying demographic and injury information. Specifically, the data for each case includes information about the decedent's demographics, any medical conditions or medications that could affect bone healing, the injury circumstances, the injured cranial vault bone(s), the type of fracture (linear, depressed, comminuted, diastatic, or other), the time since fracture, surgical interventions related to the injury, when the surgical intervention occurred, and whether there was any insertion of hardware or other material. The database also includes data on each bone sample, including the specimen size, the decalcification agent (EDTA, nitric acid, hydrochloric acid), and the decalcification duration. Other accessible data includes: *in situ* radiography, contact radiography, postmortem examination photographs, and multiple photomicrographs of the four differently stained histologic slides (hematoxylin and eosin, Masson's trichrome, alcian blue hematoxylin/orange G, and Russell-Movat pentachrome). Likely, the most valuable tool in the database is the ability for users to access the photomicrographs that consist of an overall view of the sample, a detail of the fracture, and high magnification views (20X–40X) of the outer table, diploë, and inner table.

The database also provides a search tool for users who would like to find injuries with specific parameters (e.g., age of individual, time since injury, type of fracture). This feature can be used by forensic practitioners to find fractures of known age that may be similar to a case of interest.

Ultimately, this database and the attendant study will provide the tools necessary for understanding how cranial fractures heal in various age cohorts, aid in interpretation of cranial fracture healing rates, and provide a comparative database for investigators to estimate the age of a fracture for cases in which the injury time is unknown.

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#### Fracture, Fracture Healing, Database

## A121 Variation in Human Rib Failure Mechanisms in Experimental Anterior-Posterior Loading

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**Learning Overview:** After attending this presentation, attendees will have an appreciation for interdisciplinary research in skeletal trauma analysis, specifically in human ribs. Attendees will learn how to interpret failure mechanisms (i.e., tension vs. compression) in human ribs via strain data and high-speed video.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the need for interdisciplinary analyses of skeletal trauma in ribs. Increased understanding of how ribs fail in an anterior-posterior bending scenario will serve to improve skeletal trauma analyses in forensic anthropology.

Rib fractures are of significant clinical, forensic, and biomechanical interest, yet the biomechanics of rib fractures, specifically the mechanism of failure, is incompletely understood.<sup>1-5</sup> Rib fractures are associated with traumatic events, including accidents and homicides, and a more thorough understanding of the failure mechanism, through validated, experimental trauma research, could lead to increased accuracy in re-creating traumatic events and provide support for scientific testimony.<sup>6</sup> Bone is generally known to be weaker in tension than compression; however, previous studies have found that ribs failed more frequently in compression rather than in tension.<sup>5</sup> This study aims to determine whether the failure mechanism of human ribs, when loaded in precisely the same manner, is predictable.

Sixty-nine human ribs (14 female, 55 male) from 32 Postmortem Human Subjects (PMHS) (11–90 years old, mean=48.3 years) were loaded in an anterior-posterior bending scenario at a dynamic rate of 2m/s. Ribs were each instrumented with four uniaxial strain gauges from Vishay® Micro-Measurement along the longitudinal axis on the cutaneous and pleural surfaces at 30% and 60% of the rib's total curve length. Data directly collected and utilized in this research included: peak strain (microstrain) from strain gauges and time (millisecond). Each experimental test was recorded using a Phantom® VEO 710L high-speed video camera at a minimum of 8,000 frames per second (fps). For a comprehensive description of experimental test details and boundary conditions, see reference.<sup>7</sup>

Strain modes were as anticipated for such a bending scenario; all cutaneous gauges recorded tensile strain (+) and all pleural gauges recorded compressive strain (-). Strain data from the gauges closest to fracture location were used to identify the time of peak strain. Failure mechanism was then determined by quantifying the difference in timing of peak strain between the cutaneous and pleural gauges; whichever gauge reached peak strain earlier revealed the surface on which the bone initially failed and therefore the failure strain mode (tension or compression). Experimental test videos from each impact were viewed frame-by-frame to observe whether the failure initiated on the cutaneous or pleural surface of the bone. In cases where more than one failure occurred, only the initial failure was considered in this analysis. Video data were then compared to strain gauge data to determine whether the data collected from each method were consistent.

The strain gauge data revealed initial failure in tension ( $n=34$ ) occurring more frequently than in compression ( $n=29$ ); however, these data also found simultaneous failure in tension and compression ( $n=6$ ) that was not observed in the high-speed video data. From the corresponding high-speed videos, initial bone failure occurred more frequently in tension than compression ( $n=43$  and  $n=26$ , respectively). The consistency rate of the initial failure mechanism between the strain gauge data and high-speed video was only 47.8%, indicating that even video captured at 8,000–12,000fps may not be enough to elucidate failure strain mode (i.e., mechanism).

Overall, results indicate that a definitive statement regarding whether human ribs subjected to anterior-posterior bending always fail in tension or compression cannot be made. Bones, specifically ribs, are complex structures and likely do not respond to loading like other skeletal elements. Whether utilizing strain data or high-speed video data, ribs failed in either tension, compression, or simultaneously in tension and compression. Previous assumptions regarding the consistency of ribs failing in a specified strain mode may be unfounded. Additionally, results demonstrated that the method in which failure was evaluated affected the determination of failure mechanism. Interdisciplinary experimental research is imperative to further investigate failure mechanisms utilizing a hierarchical model (i.e., from the tissue level up to the individual level) in ribs and other skeletal elements.<sup>5</sup>

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### Rib Fractures, Fracture Mechanism, Injury Biomechanics

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## A122 Rib Fractures: An Experimental Approach to Identifying Intrinsic Sources of Variability

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**Learning Overview:** After attending this presentation, attendees will have an appreciation for interdisciplinary research in skeletal trauma analysis, specifically in human ribs. Attendees will learn how to conduct experimentally driven biomechanical validation of skeletal trauma for forensic anthropology purposes, and understand the importance of utilizing experimental data to support or challenge current assumptions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the need for interdisciplinary biomechanical analyses of skeletal trauma in ribs. Increased understanding of intrinsic sources of variability in rib failure mechanisms will serve to improve skeletal trauma analyses in forensic anthropology.

Rib fractures occur frequently in motor vehicle crashes, accidents, and homicides and, therefore, are a high priority for skeletal trauma analysis in forensic anthropology.<sup>1,2</sup> Love and Symes provided strong justification for paying closer attention to rib fractures as they noted that on a large sample of adult ribs, the observable fractures did not necessarily follow predictable patterns.<sup>3</sup> Since these data were collected on forensic case studies, there is no way to know what the actual circumstances were that led to the fractures, and, therefore, details such as fracture mechanism as well as magnitude and direction of force cannot be substantiated. They challenge the forensic anthropology community to consider the influence of bone material and structure in interpreting its mechanical behavior. Others have also emphasized the critical role in understanding biomechanics in order to effectively assess skeletal trauma.<sup>4</sup> Some attempts have been made to induce human rib blunt force trauma in controlled settings, but these generally have included only small sample sizes that cannot satisfy *Daubert* criteria.<sup>5</sup> Nonetheless, more biomechanical experiments are critical to make advances in trauma analysis in forensic anthropology. Therefore, the goal of this study was to identify intrinsic sources of variability on rib response and fracture behavior. Additionally, common assumptions related to fracture outcomes were tested.

Dynamic (1–2m/s) experiments simulating a frontal blunt chest impact conducted in the laboratory on mid-level (5–7) human ribs from donors of all ages (4–108 years) and both sexes form the basis of this work ( $n=347$ ). By creating a repeatable and tightly controlled experiment, all variance observed in how the rib responds to applied loading can be attributed to intrinsic bone properties. Three hundred ninety-one fractures occurred. In general, the most crucial finding from this research was that rib response in a simplified bending scenario may not be predictable. A rib's resistance to loading (measured by peak transmitted force) varied significantly by sex (students *t*-test,  $p<0.005$ ), but was *not* successfully predicted by age (linear regression,  $R^2=7.3\%$ ). Wedge fracture types (23%) were *not* the most common as expected; instead simple fracture types (70%) occurred most frequently. Fracture type did have a significant relationship with age (Kruskal-Wallis,  $p<0.005$ ), but *not* peak transmitted force (Kruskal-Wallis,  $p>0.005$ ). Despite a consistent applied force among tests, the number of fractures that a rib sustained (a measure of injury severity) varied from zero (0.8%), one (81%), two (16.5%), or three (1.7%) and had no relationship with peak transmitted force (Kruskal-Wallis,  $p>0.005$ ). Cross-sectional geometric properties were most influential in determining the rib's resistance to loading. For example, cortical area explained 55% of the variance in force (linear regression,  $p<0.005$ ) and should be employed in interpretation of fractures by forensic anthropologists. This complex, hierarchical approach to finding mechanistic biological explanations for rib response to traumatic loading will be discussed in detail and will help to form a thorough understanding of why and how these injuries occur.

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### Blunt Force Trauma, Injury Biomechanics, Rib Fracture

## A123 Histological Age Estimation of the Femur Using Random Forest Regression

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**Learning Overview:** After attending this presentation, attendees will be re-introduced to the histological age estimation method developed by Crowder and Dominguez in 2012, and be introduced to the use of random forest regression to generate age estimates.<sup>1</sup> Additionally, an application to perform these regressions will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a more flexible statistical approach for histological age estimation than has been previously applied, resulting in a higher quality of forensic practice and reducing errors in histological analysis.

Estimating adult age is a challenging aspect of biological profile construction. Histological methods have been touted as a more accurate approach to adult age estimation because they are based on the continuous turnover of primary by secondary cortical bone and therefore follow a more predictable rate of change. However, research demonstrates that past methods succumb to the same issues affecting gross methods. In 2012, Crowder and Dominguez introduced a method that addressed issues such as subjective variable definitions, difficulty reproducing microscopic field sizes, spatial variation of histological structures, and the statistical significance of histological variables.<sup>1</sup> They employed stepwise linear regression to evaluate the following variables: osteon population density (intact and fragmentary), mean osteonal cross-sectional area, and mean anterior cortical width. Three separate regressions, one each for females, males, and a pooled model, were performed. The female model achieved the highest  $r^2$  at 0.682 (SEE=9.64 years); males had the lowest  $r^2$  at 0.510 (SEE=11.95 years); and the pooled model was in-between with an  $r^2$  of 0.587 (SEE=11.19 years). While this method minimized observer error through improved variable definitions and accounted for a large amount of spatial variation for histological features, it failed to improve a common limitation of adult age estimation—the use of linear regression analysis.

The current study will re-evaluate the variables from the Crowder and Dominguez study using random forest regression.<sup>1</sup> Random forest regression is a flexible, non-linear method utilizing decision trees and aggregated estimates. Essentially, a forest of decision trees, of a user-defined size (typically between 100 and 1,000), is constructed in which random variables are tested at each decision node and individual estimates of age are generated; the number of estimates is equal to the number of decision trees.

The sample includes 236 (120 males, 116 females) femur cross-sections of known-age individuals from three histological collections.<sup>2,3</sup> Histomorphometric data were collected using a transmitted light microscope and a firewire camera. The topographic sampling method was modeled after Iwaniec and colleagues and Stout and Paine.<sup>4,5</sup> Using a Merz counting reticule at 200x magnification (field area=0.2304mm<sup>2</sup>), the method evaluates alternating fields in ten columns from the periosteal to the endosteal cortex at the anterior femur midshaft. This sampling strategy is designed to capture the remodeling variability within the anterior cross-section. Osteon areas and cortical widths were calculated using imaging software.

The current study utilizes the Random Forest package in R to generate three models (female, male, pooled sex) using 800 decision trees. Each tree utilizes separate, bootstrapped training and testing sets to avoid overfitting and to reduce overall bias. Individual estimates are then aggregated by taking the mean of each estimate as the final predicted age. Each individual estimate is retained and can be directly modeled for a Prediction Interval (PI). Random forest regression models, unlike linear regressions, make no normality assumptions, and, as a consequence, PIs cannot be easily generated. Individual age estimates from the model are summarized using quantiles (2.5 and 97.5) to generate a 95% PI. The  $r^2$  values for the resulting models each exceeded those obtained via linear regression reported by Crowder and Dominguez: female=0.796 (MD=3.85 years), male=0.692 (MD=4.56 years), and general model=0.719 (MD=4.17 years).<sup>1</sup> Similar to the results of Crowder and Dominguez, the most important variable in each model is fragmentary osteon population, followed by intact osteon population, cortical thickness, and, lastly, osteon area.

Random forest regression offers a flexible alternative to traditional methods without suffering from the assumptions of linear models. Random forest regression improves estimates and model fits by all available metrics and can be easily implemented with the newly developed application named keRley. This research will benefit the forensic community by generating more accurate age estimates without violating any statistical assumptions.

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### Forensic Anthropology, Histomorphology, Random Forest Regression



## A124 Longitudinal Variation in Skeletal and Dental Development

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**Learning Overview:** The goals of this presentation are to present attendees with a longitudinal comparison of skeletal and dental development, followed by an exploration of preliminary patterns in health, and their potential health implications.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the awareness that variations in development may be useful in improving the understanding of health histories of the deceased. Further, this presentation will shed light on importance sources of error in juvenile age estimation, including normal variation in the rate of development.

Skeletal and dental development are well known as being useful for the age estimation of juveniles. Complexities in age estimation arise when estimates derived from these two sources of data are dissimilar. Current research on the correlation between the systems has focused on cross-sectional correlations of sample means. In contrast, this presentation will explore the skeletal and dental growth patterns of individuals who were delayed, average, or advanced in their skeletal or dental development at the age of 3 years.

Longitudinal skeletal and dental development was estimated from the radiographs of 150 White (European American) girls who were part the Bolton-Brush Growth Study. Individuals had hand-wrist and lateral cranial radiographs taken at ages 3, 6, 9, and 12 years. Developmental age estimates were calculated using Greulich and Pyle for carpal development, and the London Atlas for dental development.<sup>1,2</sup> The sample was divided into two sets (dental or skeletal) of three subgroups (delayed, average, or advanced) based on development at age 3 years. Repeated measure Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference analyses examined the longitudinal growth patterns of: (1) skeletal development of skeletal subgroups, (2) dental development of skeletal subgroups, (3) dental development between dental subgroups, and (4) skeletal development of dental subgroups.

Information on the girls' health data had been collected by Bolton-Brush researchers via a questionnaire to parents at each visit. The questionnaire included ten specific common childhood diseases, as well as colds and diarrhea. A cumulative count of total illnesses was made for each individual. Mixed linear models were applied to the four models to investigate the significance of disease on development.

The four models demonstrated significant differences between the three subgroups. For models 1 and 3, the pairwise comparison between the subgroups found significant differences between the developmental trajectories. This was not true for models 2 and 4. For model 2, delayed versus average and average versus advanced were significant, while for model 4, only the average versus advanced comparison was significant. This has important implications for the forensic estimation of age, because it suggests a complication in the relationship between the systems that has not been adequately explored.

Individuals who were delayed dentally or skeletally were consistently those who were the sickest between the ages of 1 and 5 years old. However, mixed linear models failed to find any significant effect of illnesses on skeletal or dental development when the three subgroups (delayed, average, or advanced) were compared. Additionally, based on the consistent pairwise significance of the average versus advanced comparison, mixed linear models were applied to only the average and advanced subgroups. Again, no significant effects of illness on development were observed for any of the four models.

These results reflect differences in development of the skeletal and dental systems between those who were at average development at 3 years of age versus those who were advanced at 3 years of age. Although these analyses failed to identify statistically significant effects of health, the case of childhood illnesses affecting the growth patterns of developmental variation is far from closed. Additional approaches to analysis might include primarily focusing on earlier illness events rather than health throughout childhood and approaching variation retrospectively from age 12 rather than predictively from age 3.

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### Health, Age Estimation, Growth Patterns



## A125 Subadult Age Estimation Using a Mixed Cumulative Probit and Its Application in KidStats

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**Learning Overview:** After attending this presentation, attendees will be able to better estimate subadult age both methodologically, with the introduction of the mixed cumulative probit, and practically, with the introduction of a freely available graphical user interface, KidStats, and the R package, yada.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community as there is currently no method to build a multivariable, multi-indicator model using both continuous and ordinal data to estimate a continuous variable (i.e., age). Additionally, this presentation will introduce the virtual subadult anthropological database, which contains the largest number of de-identified computed tomography images of individuals ranging in age from birth to 20 years from around the world, housed at the University of Nevada, Reno, and available for research.

To date, positive identification of a subadult is largely dependent on age estimation. There has been a stagnation in the development and validation of age estimation methods, in part due to the lack of available subadult data in skeletal collections. In an effort to advance the forensic anthropological toolkit, as well as better understand growth and development of modern children, a virtual multiregional skeletal collection of approximately 2,500 subadults has been amassed and is housed at the University of Nevada, Reno. The North American sample comes from the University of New Mexico (UNM) Health Sciences Center, Office of the Medical Investigator (OMI) and the Office of the Chief Medical Examiner in Baltimore, MD. The worldwide samples come from Taiwan, France, the Netherlands, South Africa, Angola, Brazil, and Colombia. Supplementing skeletal collections with computed tomography images and full body X-rays has provided a unique opportunity to collect a large number of age indicators—diaphyseal dimensions, epiphyseal fusion, and dental formation—on these individuals to better understand the inherent variability of the age indicators and how it impacts age estimation.

A new algorithm, the mixed cumulative probit, was developed. It is an improved extension of the cumulative probit that handles both ordinal and continuous predictor variables to estimate a single continuous variable, deals with missing data, takes into account correlations among indicators, allows heteroscedasticity in component variables, and provides an estimate of age with 95% confidence intervals. The algorithm is available for forensic anthropologists through the user-friendly graphical user interface KidStats, and also as a separate R package, yada, so it can be employed by other researchers.

This presentation will demonstrate the performance of the mixed cumulative probit using 1,100 North American individuals aged between birth and 20 years, with different variable combinations. Cross-validation was built into model development and was applied to a hold-out sample, offering the most realistic assessment performance. The results indicate narrower confidence intervals than previously provided by any age estimation technique, ranging from 0.1 months to 5 years. The larger confidence intervals are not automatically linked to older individuals, but rather to individuals that had fewer variables input into the algorithm. Results demonstrate that combining all subadult indicators yields a more precise estimate. This study did not see any patterned bias in the age estimates, likely because the model is Bayesian in nature (and therefore has no regression to the mean effect) and because the parametric model used for inference is more flexible than previous models. Comparable performance in the training and test samples suggests there is no over- or under-fitting.

The freely available graphical user interface KidStats allows practitioners to employ this robust method in casework; it yields an age estimate given one of the predictor variables or up to the maximum number of 27 predictor variables. Additionally, the large number of individuals in model development enables the user to choose from a multitude of reference samples to use a global model, a population-specific model, or a model based on any combination of reference samples. If individuals are interested in using this algorithm to develop different estimation methods, it is also available in the yada R package.

The mixed cumulative probit will not only improve subadult age estimation but broaden methodological possibilities for adult age estimation as well. Furthermore, it has a number of applications both within and outside the field, such as paleodemography, economics, and human biology.

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### Subadult, Age Estimation, Cumulative Probit



## A126 Conditional Independence and Appropriate Number of Stages in Juvenile Dental Age Estimation

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**Learning Overview:** After attending this presentation, attendees will better understand the importance of testing for conditional independence, as well as how to determine the most appropriate number of stages in age estimation methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by challenging an assumption that impacts the correct precision of estimated age intervals.

Juvenile age estimation escapes many of the problems that plague adult age estimation by virtue of the fact that developmental characteristics are more highly correlated to chronological age than are degenerative characteristics. While juvenile age estimation is less prone to issues such as wide age intervals and age mimicry of reference samples, other important challenges are shared. The viability of the conditional independence assumption must be questioned in both juvenile and adult age estimation. This issue is even more pressing in juvenile age estimation where a much larger number of traits are often included in a single estimate.

Conditional independence means that two traits are independent of one another once the effect of chronological age on each trait is accounted for. This assumption allows for the straightforward construction of age intervals when multiple indicators are used. Unfortunately, further testing has revealed that the assumption does not hold for either adult or juvenile age indicators.<sup>1</sup> Assuming independence of related indicators will produce intervals that are too narrow. Furthermore, this raises the possibility of bias, since the number of indicators used may not be large enough to support the central limit theorem, especially when indicators are not independent. This project tests the hypothesis that the permanent dentition within a single quadrant are conditionally independent from one another in development given age.

The data consist of Moorrees, Fanning, and Hunt scores of the lower left quadrant of permanent dentition from panoramic radiographs of 738 female British children between 2 and 25 years of age.<sup>2</sup> Data were generously donated by Helen Liversidge. Ten percent of the sample ( $n=74$ ) was withheld from analysis for cross-validation. Every stage for each tooth was tested for outlying ages using a first derivative test. Lagrange multiplier goodness-of-fit tests were used to determine whether the data fit a cumulative probit model on a straight or log scale. For teeth that did not fit the model with fewer than 1% of the training sample removed as outliers, stages were systematically collapsed until a fit was found. Both staging systems were then tested for conditional independence using the mvord package in R.<sup>3</sup> The collapsed stage system was compared to the original staging system by estimating ages for the withheld portion of the sample via the corresponding cumulative probit models.

All but the third molar fit a log-scale cumulative probit model with outliers removed. For the third molar, stages 0-2 were collapsed, producing a 14-stage model. Variance-covariance matrices of the original and collapsed staging systems produced chi-square values of 763 and 1,296, respectively, when compared to the identity matrix using Bartlett's test. The  $r^2$  between known ages and estimated ages of the withheld sample was 0.9255 for the original staging system and 0.9267 for the collapsed staging system. Slopes were 0.9611 and 0.9595 respectively. When 95% probability regions were constructed for the withheld samples, the actual success rate of true ages falling within the estimated interval was 72.9% for both systems.

In conclusion, the collapsed staging system performed similarly to the original system in accuracy and precision of estimates as well as conditional independence of teeth. This indicates that it is possible to optimize dental staging systems so that only meaningful transitions are recorded. Low success rates indicate that the conditional independence assumption is not supported in either case. Failure of this assumption produces age intervals that are too narrow.

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### Juvenile Age Estimation, Dental Development, Conditional Independence

## A127 Dental Morphology as a Key to Understanding the Population History of Latinos

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**Learning Overview:** After attending this presentation, attendees will better understand the utility of dental morphology in exploring the ancestry of modern Latinos through their population history.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how dental morphology can augment information provided by genetic, cranial, and historical studies, especially as they pertain to groups with diverse population histories resulting in clinal variation, such as Latinos.

The modern Latino population has been established over the past 500 years through genetic drift and gene flow events resulting from European colonization of the New World. Existing variation among Native American, European, and African populations that are parental to Latinos has created a mosaic of genotypic and phenotypic variation that is only beginning to be understood within a forensic anthropological context. As Latinos are among the fastest growing populations within the United States, it is critical that every available tool be utilized to explore their variation so ancestry estimations can continue to improve for existing populations within this country.<sup>1</sup> Genetic studies of Latino populations within the United States show that they generally fall into either a dihybrid or trihybrid pattern of ancestral influence, with levels of parental ancestry varying regionally.<sup>2-5</sup> Regional trends within the United States and Mexico are well-known and have been supported with cranial studies.<sup>6-9</sup> Dental morphology studies exploring this variation, however, are rare, despite moderate to high levels of heritability associated with tooth shape.<sup>10-13</sup> This study examines dental morphology from pre- and post-contact, as well as forensically significant populations, to gain insight into how microevolutionary forces have affected the modern Latino population. The primary goals of this preliminary investigation are: (1) to explore the variation within Native American populations, historic European/American, African/American, and Latino populations, and (2) to see how these populations may have contributed to a modern Latino population.

Samples within this study include: Native American data from Mexico City from the Escuela Nacional de Antropología e Historia (ENAH) and samples from the Christy G. Turner II Database (with permission and access granted by Dr. G. Richard Scott), and a New Mexico sample from the Phoebe A. Hearst Museum of Anthropology; historic European and West African data from the Turner II Database; historic Latino sample from ENAH; historic European American and African American data from the Robert J. Terry Collection; modern European American data from the Texas State University Donated Skeletal Collection and the Documented Skeletal Collection at the Maxwell Museum at the University of New Mexico; and a modern Latino sample from Mexico City at the Universidad Nacional Autónoma de México (UNAM). Morphological data from the Turner II Database were collected according to the published Arizona State University Dental Anthropology System (ASUDAS) standards and data collected for this study were gathered according to the new ASUDAS standards.<sup>14-15</sup> Previously conducted intra-observer error tests found trait agreement at levels of 0.621 or above.<sup>16</sup> Pearson's chi-squares were applied to raw scores for 27 dental morphological traits, with all but two differing significantly ( $p < 0.05$ ) among the samples. The remaining traits were subjected to a Kendall's tau to examine inter-trait correlations; traits with correlation values of 0.4 or higher or those with an uncertain key tooth were removed from future analyses. Additionally, Pearson's chi-squares were conducted to check for sex-correlated traits within the samples. The remaining 19 traits were dichotomized for Mean Measure of Divergence (MMD) and Fuzzy C-Means (FCM) analyses. MMD results show some significant variation among the Native American samples, the historic samples all differ significantly from one another, and the modern Latino sample differs significantly from most samples, except for the New Mexico Native American sample and the historic Latino sample. The FCM analysis shows much overlap among the samples, with only the Texas State sample not falling in the largest cluster with all other samples.

These exploratory results demonstrate there is overlap among the samples through time and space, though some notable differences are present among the modern and pre-contact samples that warrant further analyses. Dental morphology for Latinos, however, does align with genetic and cranial data showing expected influences based on population history with Native American, European, and African ancestries. The continuation of this project will include additional samples and classificatory statistics for application within a forensic setting.

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### ASUDAS, Ancestry, Latino



## A128 Cranial Feminization Surgery Methods and Osteological Identification of Post-Operative Individuals

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**Learning Overview:** After attending this presentation, attendees will understand skeletal alterations that manifest as part of cranial feminization surgery.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing details about cranial feminization surgery in general, and the resulting skeletal and soft tissue modifications specifically. Further, this presentation outlines the alterations that can be expected on the skull of post-operative individuals with variable healing (i.e., bone remodeling and modeling).

The number of transgender individuals in the United States has been estimated between 0.6%, or 1.4 million people, (through the Williams Institute in 2016) and 3% of the population, or 7 million people (by GLAAD in 2017). This group experiences high rates of violence, with 165 trans people murdered in the United States between 2008 and 2016.<sup>1</sup> Therefore, there is a growing need for the forensic community to be able to recognize the signs of cranial feminization surgery to facilitate a positive identification. Considering best practices for sex estimation in forensic anthropology specify observations of the pelvis and cranium, it is especially important for forensic anthropologists, and other individuals involved in constructing a biological profile, to be aware of the signs of surgical cranial feminization.

This presentation begins with a review of craniofacial surgical feminization techniques first developed in the 1980s. An examination is then provided of pre- and post-operative radiographs and Computed Tomography (CT) scans of 18 patients that underwent male-to-female cranial cosmetic surgery. Post-operative radiographs and CT images ranged in generation from 5 to 20 months following surgery. Four anatomical areas are identified on the skull that contribute to the accurate identification of post-operative individuals: (1) reduction of the glabella, (2) shortening of the nasal bones, (3) vertical reduction of the chin, and (4) alteration to the gonial angle (i.e., lateral flaring of the mandible).

In all patients, surgical alteration was made to these four anatomical areas of the skull to varying degrees. In 86% of cases, reduction of the glabella is achieved via osteotomy, in which the glabella is repositioned and the anterior wall of the frontal sinus reconstructed.<sup>2</sup> Most individuals in the sample (94%; 16/17) had at least a small area of anterior wall of the frontal grafted with steel wire. Nasal bone reduction was achieved through removal of the inferior nasal bone below a transverse incision and cartilage augmentation. Reduction of the chin height was achieved via osteotomy and fixation of a reduced mental eminence with titanium hardware in 94% (16/17) of the sample. Mandible alteration typically consisted of burring the outer cortical bone of the most lateral portions of the mandibular body. In 53% of the sample, the reduction resulted in exposure of the trabecular bone.

While the post-operative images range between 5 and 20 months and all patients have functionally healed, no images show complete remodeling of the bone. Patients of cranial feminization surgery continue to show ridges, or “bone scars,” on the frontal bone and mandible even after the bone is inactive. Because the remodeling process may never obliterate evidence of the skeletal procedures, the modifications could provide evidence to facilitate positive identification long after the surgeries were performed.

In addition to skeletal alterations, changes to the soft tissue were documented. These include feminization of the hairline, upper lip augmentation, and reduction of the thyroid cartilage. None of these surgeries were visible on the skeleton and would not necessarily aid in the identification of greatly decomposed individuals.

As these surgeries are increasingly practiced in the modern population, it is imperative that the forensic field can identify and appreciate signs of gender confirmation surgery in skeletal remains.

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### Cranial Feminization, Sex Estimation, Surgery



### A129 A Classification System of Thermal Damage to Human Remains

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**Learning Overview:** This goal of this presentation is to provide a comprehensive and easily interpretable classification system for the degree of thermal damage to human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how a comprehensive classification system will facilitate discussion between researchers and practitioners and provide a realistic assessment of the types and scale of damage seen. Descriptions about the level of damage and the most critical observations that should be made on a fire victim are addressed.

Classification of thermal damage to human remains is lacking the specificity that comes with witnessing multiple fire-related experiments over a wide range of settings. Currently available classifications are relatively gross and are derived from burn situations where calcination and fragmentation were the norm. This presentation outlines a classification based on 12 years of experience with the San Luis Obispo Fire Investigation Strike Team's Forensic Fire Death Investigation Course (FFDIC). The system is based on changes to: (1) soft tissue; (2) exposure of body components (fat, skeletal muscle, internal organs, bone); (3) changes in body position; and (4) skeletal damage from discoloration to calcination.

The classification system utilizes the normal progression of changes to the human body as both fire temperature and duration increase. The ten stages are developed from "minimal burning" to "calcination and fragmentation." Assignment can be made by whole body and by body segment. The classification chart includes lateral images of a body along with descriptions of soft tissue, body position, and skeletal changes.

In this initial study, inter-observer error is assessed between two people with extensive FFDIC experience and between the FFDIC people and a practicing Forensic Anthropologist (FA) who is familiar with burn cases but without as extensive fire progression experience. Twenty-four cases were selected from the FFDIC records from 2010 through 2014. All photographs of the body were placed in a blind sample file. These were assessed by each person separately. FFDIC inter-observer agreement was 75% for exact match and 100% within one stage. Between the FFDIC people and the FA person, 60% of cases were classified as an exact match and 83% within one stage. Difficulties arose in assessing images in which not all the body was visible or where differential burning was significant.

Variation in degree of heat-related changes is due to a number of factors. Bodies often do not exhibit full pugilistic changes due to the surrounding or overlying materials or differential heating. In such circumstances, the arms are most often affected, where muscle mass may be less. Proximity to the major heat source is also different within structural fires. Those areas further from the center of the fire may be less affected than the areas of the body where temperatures are more extreme. Body build is another factor, with thicker muscle and fat layers protecting internal organs and bones. Finally, it is critical that assessment be made either at the scene or from photographs taken at the scene due to the major changes brought about by transportation. Regardless of the factors, a common and comprehensive classification system that abides by the normal progression of changes due to fire is an asset.

#### Fire, Thermal Damage, Classification



## A130 Quantitative Ancient and Forensic DNA Techniques for Maximum DNA Recovery From Thermally Altered Bones and Teeth

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**Learning Overview:** The objective of this presentation is to convey the success of obtaining highly degraded DNA from thermally altered skeletal material. Here, the goal is to educate the attendees on the implications of applying ancient DNA methods to traditional forensic cases, and the possibility of reconstructing partial Combined DNA Index System (CODIS) Short Tandem Repeat (STR) profiles using mini-STR multiplexes, as well as reconstruct whole mitochondrial genomes using Next Generation Sequencing (NGS) technologies and bioinformatics pipelines.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering the best DNA methods used to test the quality and quantity of DNA in highly burned skeletal remains.

The recovery and analysis of DNA from burned human remains represent some of the most challenging cases in medicolegal investigations. The rapid degeneration of DNA often impedes the ability of forensic examiners to obtain adequate DNA profiles for subsequent genotyping and identification. In this study, the goals were to assess the utility of two different extraction methods used by forensic and ancient DNA researchers to obtain degraded DNA, as well as to establish the effects of heat-induced color change and type/location of element sampled on DNA quality and quantity.

Over a period of two years, this study obtained and documented 62 samples from 26 fire death and cremation cases in collaboration with the Maricopa County Office of the Medical Examiner. Samples were chosen with the goal of obtaining different skeletal tissues showing different levels of burning (levels I–V). After processing the bone to powder, each sample was divided for extraction using either the ancient DNA protocol or the forensic protocol. The total nuclear DNA concentration of these extracts was qualified with an Applied Biosystems® Real-Time Polymerase Chain Reaction (RT-PCR) using the Quantifiler® Trio DNA Quantification Kit, and STR detection was performed using the PowerPlex® ESX 17 Fast Systems STR kit by Promega®. In addition, sample extracts were also converted into double-stranded DNA libraries and enriched for mitochondrial DNA and nuclear Single Nucleotide Polymorphisms (SNPs) for next-generation sequencing.

The results show that average DNA yields recovered from fire-related victims show a progressive decrease in DNA concentration across burn categories I–V. Both extraction protocols work equally well for burn categories I–III; however, highly incinerated remains (categories IV–V) produced better CODIS STR results using the ancient DNA protocol. Overall, the data confirm the difficulties in obtaining DNA from burnt skeletal material using conventional RT-PCR and STR genotyping technologies. Confounding limitations inherent in both ancient and forensic DNA research, such as small sample sizes, intra- and inter-sampling differences in material input, low DNA yields paired with high degrees of DNA degradation, continue to restrict the amount of genetic information gleaned from the most challenging forensic cases. All these factors were recognized in this study. However, integration and adoption of optimized ancient DNA protocols, such as the Dabney DNA extraction protocol, in forensic case analyses may improve the success rate of acquiring adequate STR profiles to identify fire victims in circumstances in which traditional applications have failed.

### Forensic DNA, Ancient DNA, NGS Analysis



### **A131 Assessing DNA Quality and Quantity From Cadaveric Blood Stored on Untreated Blood Cards: The Impact on Short Tandem Repeat (STR) Quality and the Utility of Variably Amplified Markers for the Individual Estimation of Trihybrid Ancestry and Admixture Proportions**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of storage options for cadaveric blood samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing detailed information on the quality of DNA provided by storage on blood cards of cadaveric blood samples for STR typing.

Blood cards are widely used for long-term body fluid (e.g., blood, saliva, etc.) storage for DNA analysis. They are especially useful for DNA sample collection at forensic anthropology research centers, given their straightforward handling and sampling protocols, stability in ambient temperatures, small storage footprint, and minimal financial investment. However, little is known about the long-term value of blood cards in forensic anthropological research and forensic genetic casework. This study explores the quantity and quality of DNA extracted from postmortem blood samples, in terms of DNA preservation and STR typing success. Collected as part of a body donation program workflow, these blood samples were taken upon donor intake and stored on FITZCO<sup>®</sup> untreated (non-FTA<sup>®</sup>) blood cards. The study evaluates these cards in terms of DNA preservation and typing success and tests the effect of age of the blood card versus their success in amplification of STRs. As the gold standard in forensic genetics, STRs are used for forensic identification and as potential markers for global ancestry and admixture estimation. Degraded samples, including those stored on blood cards, can result in reduced STR markers sets and, in turn, compromised analyses. This study assesses these blood cards, therefore, with special consideration given to profile matching for positive identification and ancestry estimation for biological profile estimation.

In this study, the degree of DNA degradation is quantified in terms of both the amount and fragment sizes of the individual templates, testing for disagreement in genetically determined and reported sex, and evaluation of the forensic genetic typing potential of the DNA by evaluating Combined DNA Index System (CODIS) profiles generated for each case using evidence of allelic drop/in out, degradation curves, and relative fluorescent units as assessment criteria for 20 blood card samples. This study analyzes the impact of the DNA template on ancestry and admixture estimation, offering insights into the impact of degradation on population identifiability.

While STR profiles were successfully generated for most samples, the results indicate length of storage and time interval between date of death and sample collection have an impact on DNA quantity and quality of DNA, in terms of typing success. There is a statistically significant decrease in Relative Fluorescent Unit (RFU) values with increasing time interval between date of death and collection, indicating degradation in the blood card samples related to the postmortem interval prior to sample collection. The STR profiles generated were used to estimate ancestry and admixture using the software program STRUCTURE, demonstrating utility of the markers beyond individual identification purposes.

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#### **DNA, STR Typing, Blood Cards**

### A132 Postmortem Submersion Interval (PMSI) Estimation From the Microbiome of Bone in a Freshwater River

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**Learning Overview:** After attending this presentation, attendees will understand how changes in microbial communities across Accumulated Degree Days (ADD) can be used to predict the long-term PMSI for skeletal remains recovered from a freshwater river.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing proof of concept concerning a novel area of research, the use of microbial succession on skeletal remains submerged in a freshwater river to predict PMSI.

For victims who die in and/or are recovered from water, PMSI estimation has been limited to instances when invertebrates have been found on remains or when the victim was last seen alive, making it applicable in cases of known accidental water deaths only, rather than deposition of homicide and unidentified/unknown victims in water. Since the 2014 National Institute of Justice (NIJ) Forensic Science Technology Working Group highlighted the use of metagenomics to aid investigative leads and the advancement in technologies (i.e., next generation sequencing and bioinformatic pipelines), researchers have explored the use of microorganisms to predict time since death on land and in water, respectively Postmortem Interval (PMI) and PMSI.<sup>1-3</sup> These studies have demonstrated that microorganism colonization of remains occurs in a successional manner, taxa increase over the range of decomposition, and both can be used to predict PMSI. Unfortunately, these studies were conducted over short periods of time, with small sample sizes and repeated disturbance, which are knowledge gaps addressed in this study.

From November 2017 to November 2018, fresh pig (*Sus scrofa*) bones ( $N=125$  rib and  $N=125$  scapula) were submerged in cages that were attached by a rope to the wharf at the Virginia Commonwealth University (VCU) Rice Rivers Center, located on the James River in Charles City, VA. Water quality parameters were continuously and remotely logged using a permanently deployed sonde. Every 250 ADD, calculated using a 0°C threshold, five ribs, five scapulae, and 500mL of water were collected, photographed, and stored at -80°C or 4°C until processed. Water samples were filtered using a cellulose membrane filtration system; bone samples were pulverized using liquid nitrogen in a mortar and pestle. Both filters and bone powder underwent DNA extraction via ChargeSwitch® gDNA Plant Kit. If necessary, samples were purified with the Dneasy® PowerClean Pro Cleanup Kit. Library preparation followed the Kozich et al. protocol.<sup>4</sup> Specifically, 16S recombinant DNA (rDNA) variable region 4 sequencing-by-synthesis was performed on the Illumina® MiSeq® FGx2X. The resulting data was analyzed and visualized using the MiSeq® mothur SOP, mothur version 1.35.9, and R studio.<sup>5</sup>

Preliminary analyses indicated significant differences in bacterial communities among sample types (i.e., rib-scapula-water-mud). For bone types, differences across Accumulated Degree Days (ADD) were present at the phylum-level. Operational Taxonomic Units (OUT) -based beta-diversity (Bray-Curtis) suggested significant differences in ordinated space between ADD for both scapula and rib samples. Meanwhile, alpha-diversity (Shannon) indices demonstrated a curvilinear relationship with ADD. Overall, these results suggest that patterns in bacterial succession can be used to develop a PMSI estimation model.

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#### PMSI, Freshwater River Bone, 16S rRNA Gene





## A133 The Influence of Water Current on the Rate and Pattern of Decomposition

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**Learning Overview:** After attending this presentation, attendees will have an awareness and understanding of how lentic (still) and lotic (moving) water environments affect the rate and pattern of decomposition observed in submerged remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the accuracy of Postmortem Interval (PMI) estimations and furthering current knowledge of how a cadaver moves through the water column, which will prove useful for search and recovery teams in missing persons cases.

Worldwide, there are on average 360,000 water-related deaths recorded each year, making drowning the third-leading cause of death by “unintentional injury.”<sup>1</sup> However, experts believe this figure to be greatly underestimated, as the number of fatalities resulting from natural disasters and the ongoing migrant crisis in the Mediterranean are significantly underreported. Despite these statistics, there is a lack of controlled research investigating the environmental variables that impact the rate and pattern of decomposition in water. The goal of this research was to determine whether water current or turbulent flow influences the pattern and/or rate of decomposition observed in carrion submerged in water.

The duration of the experiment was 79 days in the summer of 2018 and involved the placement of six pigs (*Sus scrofa*), of 30kg average weight, into individual tanks containing 2,000 liters of fresh water. In three of the tanks, two water circulators were placed at opposing sides. This created a current, or turbulent flow, which simulated a lotic (moving) water environment. The three remaining tanks were left to stand still and represent a lentic (still) water environment. Three times a week for the duration of the experiment, decomposition was scored using the Heaton et al. method<sup>2</sup> and both normal and infrared (using a Fluke tiR125 camera) photographs of each pig were taken.<sup>2</sup> In addition, insect activity and the position of each cadaver in the water column were noted. Ambient temperature was recorded hourly by two data loggers onsite; water temperatures were recorded hourly by a data logger in each individual tank. Temperature data were also collected from a local weather station for comparison.

Statistical analyses showed there were no significant differences in the rate of decomposition when comparing the pig carcasses in the lentic water environments with those in the lotic water environments ( $p \leq 0.32$ ). This supports the conclusions made by Heaton et al that decomposition of human remains does not differ significantly among aquatic environments in the United Kingdom.<sup>2</sup> All six cadavers showed similar patterns of decay, although pigs in the lotic water appeared to disarticulate and sink somewhat faster. Water temperatures were significantly warmer than those recorded by both ambient loggers ( $p \leq 0.001$ ) and the weather station ( $p < 0.001$ ) by an average of 3.18°C. This highlights the importance of using the water temperature, rather than ambient when estimating PMI.

When initially placed in water, two of the pigs remained floating while four were immediately submerged. By day 6 (105 ADD), all of the submerged pigs had bloated sufficiently enough to resurface. Knowledge regarding the timing of resurfacing events has implications for search and recovery methods, allowing search personnel to determine where a victim is likely to be positioned in the water column so they can plan accordingly. Floating cadavers were heavily colonized by maggot masses between days 12 (219 ADD) and 25 (474 ADD), which generated enough heat to produce a discernible thermal signature, peaking at 55.6°C on day 24 (454 ADD). These results suggest there is a window of time where thermal imaging could potentially be used as a tool to locate a missing person believed to be in water. By day 30 (590 ADD), all cadavers had begun to sink, which would indicate more appropriate search techniques, such as divers or geophysical methods, would be best utilized.

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### Taphonomy, Freshwater Decomposition, Thermal Imaging



### **A134 Macroscopic Analysis and Scanning Electron Microscopy (SEM) of Immature Permanent Molars Immersed in Hydrochloric Acid (HCL, 38%)**

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**Learning Overview:** After attending this presentation, attendees will understand the importance of expanding current studies of acid disposal to include immature, incompletely developed permanent teeth. Immature permanent third molars are typical to adolescents or young adults over the age of 15 years, thus studies contributing to current literature regarding acid disposal of dental tissues from this age group are relevant to the forensic sciences.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community in terms of competence by contributing to the comparative body of knowledge regarding immature dental tissues where acid was used in the means of disposing human remains.

In this study, morphological differences in a sample of immature permanent molars before and after immersion in HCL, 38% were evaluated macroscopically and using SEM with the goal of contributing to the body of literature regarding identification of dental tissues partially or nearly completely destroyed using acid. Four intact and non-carious immature, or incompletely developed, permanent third molars were selected as the sample for this study. The teeth used in this study have roots that are not completely formed, where the inferior portion of the root can be characterized by an open apex. The walls of the root are also much thinner than completely developed permanent molars. Incompletely developed permanent third molars are typical of adolescents or young adults over the age of 15 years. The teeth comprising this sample were extracted during the course of normal orthodontic practice and made available for educational purposes. The teeth were sterilized within a week of extraction using an autoclave (121°C, 15lbs psi). Standard odontometric measurements, weight, photographs, and imaging using a Nanoimages SNE-3200M mobile SEM were taken for each tooth before and after immersion in a commercially available formulation of HCL, 38% within brief (1–8 hours), moderate (8–16 hours), and extended (over 16 hours) time intervals. After treatment, the teeth were dried, remeasured, and reimaged using the mobile SEM.

The results of the macroscopic analysis show the sample teeth exposed to acid for a brief duration exhibited transparency in color, gelatinous texture, minor changes in morphology of the crown, such as sharpening of the cusps, reduction in amount of enamel visibly present, and reduction in size of the tooth as measured using standard dental measurements and weight in grams. These changes intensify at the moderate and extended time intervals and are presented with SEM imaging. Microscopic morphological differences in the dental tissues are highlighted.

A dearth of literature exists describing the effects of acid on teeth using SEM imaging. This is especially true concerning adolescent teeth. However, the existing literature does establish acid immersion as a method of body disposal. When suspected human remains are found in acid, limited information is available to assist investigators in determining whether human remains are, in fact, present. Because teeth are the strongest part of the human body, they are more likely than other human substances to be found in a solid state. Teeth represent the most likely human tissue to aid in an investigation where acid is used as a means of body disposal. This research provides academics and practitioners new information regarding time estimates for human remains in acid as well as the ability to identify when HCL was used in the event the acid was strained.

#### **Body Disposal, Hydrochloric Acid, Immature Dentition**

## A135 A Paired Comparison of the Rate and Pattern of Decomposition in Small- and Large-Bodied Human Cadavers

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**Learning Overview:** After attending this presentation, attendees will have learned that differences in body mass of human cadavers can affect the gross morphoscopic observations of the decomposition process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the first closely paired body weight study using human cadavers to examine the difference in the rate and pattern of decomposition between small- and large-bodied individuals.

Several previous studies have examined the effect of body mass on the rate and pattern of decomposition using both human cadavers and animal proxy samples.<sup>1-9</sup> The results have proven to be inconsistent and categorically diverse. Summarily, inter-cadaver decomposition rate studies categorically suggest: (1) larger individuals decompose faster; (2) there is no difference in the rate or pattern of decomposition between individuals of different sizes; (3) the inter-individual progression of decomposition varies depending on the stage of decomposition observed; or (4) some smaller individuals decompose faster than larger individuals.<sup>1-12</sup> Studies that empirically test body mass as an important variable in human decomposition have largely been conducted using human analogs. Only two of these studies have used human cadavers and considered the longitudinal postmortem intervals that characterize forensic anthropology casework.<sup>1,7</sup> However, these studies diverge in their conclusions. Mann and colleagues suggest larger individuals decompose faster than smaller individuals as a result of the rapid liquefaction of body fats, while Roberts and colleagues demonstrated no correlation of body mass to Kelvin Accumulated Degree Days (ADD) at any observation threshold representing early, middle, and late stage decomposition, although minor differences in the pattern of decomposition were noted.<sup>1,7</sup> In both of these previous studies, individuals were observed independently of the larger project, and data were collated retrospectively.

This study focuses on the paired observation of two individuals at the Complex for Forensic Anthropology Research (CFAR) at Southern Illinois University in Carbondale, IL. The two individuals observed died on the same day (May 17, 2019), were close in age at death (67/68 years old), and were refrigerated in funeral home coolers for the same length of time (seven days) at the same temperature (37°F). They were placed at CFAR on the same day, within 15 minutes of one another. The larger individual is an adult male, 188cm (6'2") in height, weighing 125kg (275lbs), with an estimated Body Mass Index (BMI) of 35.5 at the time of death. The smaller individual is an adult female, 150cm (4'11") in height, weighing 44kg (97lbs), with an estimated BMI of 19.6. Both weights were confirmed by the funeral home contracted to deliver the cadaver. Decomposition was categorically scored using the Megyesi et al. Total Body Score (TBS) system; macroscopic observations not represented in the TBS model were also described.<sup>13</sup> Digital photographs were collected every third day, unless inclement weather interfered with camera use. The decomposition of the two individuals rapidly diverged, with the larger individual advancing more rapidly through the initial stages of decomposition, including color change, bloat, and soft tissue loss throughout the first 23 days post-deposition, suggesting gross decomposition was more rapid. After 23 days of exposure (more than 517 ADD), the advancement of TBS slowed and the two individuals scored nearly identically ( $\leq 1$  TBS point difference) for the next 44 days (until July 29, 2019). Further data will be collected over the course of the next six months. However, in sum, this paired-individual study suggests that larger individuals initially decompose faster than smaller individuals, but as decomposition advances, the differences are marginal. This study also demonstrates the differences in the pattern of decomposition between larger and smaller individuals, which is consistent with previously reported results from varying sources.

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### Forensic Taphonomy, Forensic Anthropology, Total Body Score

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### A136 The Differential Rate of Human Decomposition in an Enclosed Vehicle Compared to an Outdoor Environment

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**Learning Overview:** After attending this presentation, attendees will understand the difference in the postmortem interval between human cadavers decomposing inside a vehicle and control cadavers in similar body positions but located outdoors. Attendees will learn about the factors contributing to the faster rate of decomposition in the control cadavers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting new postmortem interval data, including factors that affected the rate of decomposition between the two environments and the differences in gross appearance throughout the decomposition process.

There is controversy within the literature regarding different rates of decomposition and whether decomposition occurs faster or slower in indoor or outdoor environments. Voss' study, using pig carcasses, showed that carcasses inside a vehicle decomposed faster than the outdoor carcasses due to environmental factors, including higher temperatures in the vehicle.<sup>1</sup> Because decomposition varies between humans and animals, the present study explored if this would be true for human cadavers. This study compared the decomposition rate of two cadavers in a closed vehicle, with two control cadavers in an outdoor surface environment. The cadavers in the vehicle were placed with one in a sitting position in the driver's seat, and one in a loosely flexed position in the trunk. Two control cadavers were placed in similar body positions outdoors. Photos were taken daily until the advanced decomposition stage was reached, then taken weekly thereafter. Physical descriptors for southeast Texas, developed by Bytheway et al. were used to estimate the stage of decomposition for each cadaver.<sup>2</sup> Results showed that the control cadavers (CAD 3 and 4) went through the fresh and early stages of decomposition faster than the experimental cadavers (CAD 1 and 2). CAD 1 was in the driver's seat of the car and CAD 3 was in an upright position in a chair outdoors. For the Fresh stage of decomposition, CAD 3 progressed one day faster than CAD 1. CAD 3 also decomposed faster in the Early stage of decomposition by three days. A similar trend was observed with the trunk control (CAD 4) decomposing faster in the Fresh stage by 1.5 days compared to CAD 2. In the Early stage, CAD 4 decomposed approximately 2.75 days faster than CAD 2. The experimental cadavers remained in the Advanced stage of decomposition for the remainder of the study (365+ days). The control cadavers took approximately 152 days to reach skeletonization.

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### Forensic Taphonomy, Human Decomposition, Vehicle



## A137 Location, Location, Location: Environmental Variation and Human Decomposition in Knoxville, Tennessee

William D. Cawley, MA\*, University of Tennessee, Knoxville, TN 37920-2605; Derek A. Boyd, MA, University of Tennessee, Anthropology, Knoxville, TN 37996

**Learning Overview:** After attending this presentation, attendees will better understand how environmental variation affects human decomposition at the University of Tennessee Anthropological Research Facility (ARF) in Knoxville, TN.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by investigating a potential confound affecting research within a single microclimate that involves the estimation of the Postmortem Interval (PMI) using human subjects. Attendees will increase competency in understanding how environmental variation affects the rate of decomposition and increase performance by assisting future research designs of human decomposition studies.

The estimation of the PMI *vis-à-vis* gross evaluation of human decomposition is an essential aspect of medicolegal death investigation. Anthropological research often assumes individuals within the same microclimate will display similar rates of decomposition.<sup>1</sup> Underlying past studies is the assumption that minor variations in location within these microclimates have a negligible effect on the rate of human decomposition and that only large-scale climatic differences affect the estimation of the PMI. The ARF is a major center of human decomposition research and has produced studies involving the determination of PMI using gross human decomposition since the 1980s. Expansions to the ARF, now encompassing an area of approximately three acres, have facilitated projects that incorporate PMI estimation using multiple donors located throughout the property. The ARF land consists of multiple zones that vary by soil type, drainage, elevation, ground foliage, tree density, and use rates, making it a valuable study facility for environmental variation in human decomposition.

Image data from the Daily Photo Collection curated by the Forensic Anthropology Center (FAC) that were collected between 2011 and 2019 were analyzed to determine differences in the rate of human decomposition at the ARF. Using Geographic Information System (GIS) data maintained by the FAC, the ARF was divided into four regions using natural and artificial features of the property that affect watershed and elevation. Photographs of donors ( $n=8$  donors, approximately 24 photos per donor) located in each region were examined to retrospectively assign Total Body Scores (TBS) for the date of placement, on day 5, and on day 15.<sup>2</sup> TBS data were recorded for donors placed in these regions according to season of the placement date (total  $n=128$ , 8 donors scored per region per season) and evaluated using a Bayesian hierarchical linear model in R to characterize variation in decomposition rates between regions and across seasons.<sup>3</sup> A preliminary model using a sub-sample of donors ( $n=24$ ) from across three sectors of the ARF (B, E, and G), areas originally allocated by the FAC for daily operations, were scored at day 15 after surface placement in the late summer (July–August). Results show that the modal parameter values describing the affect of region on TBS score probabilistically differs between regions ( $\beta_B=-1.49$  [95%CrI:-4.97-1.88];  $\beta_E=2.04$  [95%CrI:-2.96-6.89];  $\beta_G=2.44$  [95%CrI: -2.18-7.27]), suggesting the existence of spatially variable environmental differences in the rate of decomposition within the ARF. Macroscopic differences regarding sun exposure proportion, foliage density, and ground-level vegetation are also explored, given the similarity and differences of these factors between areas.

This proof-of-concept study highlights the need for careful selection of areas for donor placements within microclimates (specifically outdoor research facilities) when designing research involving PMI or gross human decomposition. More broadly, the results of this study point toward the existence of environmental heterogeneity in the extrinsic and intrinsic factors long known to affect rates of decomposition.

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### Human Decomposition, Outdoor Research Facilities, Bayesian Hierarchical Model



### A138 A Preliminary Decomposition Study Within the Willamette Valley (WV) of Oregon: Multi-Method Comparison and Sharp Force Trauma Effects

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**Learning Overview:** After attending this presentation, attendees will understand: (1) how decomposition rates within the WV compare to previous research conducted in the region; (2) how Anderson's and VanLaerhoven's stages of decomposition model compares to Megyesi et al.'s Total Body Scoring (TBS) system in Oregon; and (3) how the increase in the number of open wounds (sharp force trauma) influences decomposition rates within the study environment.<sup>1,2</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the data available on decomposition, taphonomic variables, and Postmortem Interval (PMI) estimation in an understudied region.

Determining time since death (the PMI) is an essential part of medicolegal death investigations. PMI can give investigators important information about time of death and may help answer questions about the events leading up to death.<sup>3</sup> The purpose of this study was to collect decompositional data from an understudied region (Oregon) and compare multiple scoring methods that are current standards developed in regions such as Tennessee in order to characterize the effects of regional variation on decomposition and taphonomy. Six pig heads were placed on the ground surface in a fenced enclosure and exposed to the natural winter environment of the WV of Oregon for 60 days. Three of these pig heads underwent Sharp Force Trauma (SFT) infliction in order to compare rate of decay with remains that have a singular SFT wound. Stage of decomposition, temperature, precipitation, and preliminary entomological data were collected throughout the 60-day observation period. These data were used to compare Anderson's and VanLaerhoven's stages of decomposition model to Megyesi et al.'s TBS system in the WV; compare and contrast similar studies from different seasons within the WV; and analyze the effects of an increase in the number of open wounds (SFT) on decomposition rates and insect activity.<sup>1,2</sup>

This study found that decomposition in the WV during the cold/wet season (winter) did not closely align with either Anderson and VanLaerhoven or Megyesi et al.<sup>1,2</sup> Analyses of statistical, qualitative, and inter-observer error suggest that neither scoring method is a perfect fit for the WV. Winter decay was found to occur at a slower pace when compared to summer decay and was overall more variable. Partial mummification and rehydration of the remains were observed multiple times during this study. An increase in the number of SFT wounds did not influence the rate of decay. Sub-environmental differences were found to have an effect on decomposition rate, and a considerable amount of small animal and avian scavenging of the remains occurred throughout the study. Scavenging influenced the rate of decay through loss of mass that propelled decomposition forward.

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#### Taphonomy, Oregon, Decomposition



## A139 Evaluating the Application of Multiple Postmortem Interval (PMI) Estimation Methods in Louisiana

Sophia I. Reck, MA\*, Baton Rouge, LA 70806

**Learning Overview:** After attending this presentation, attendees will better understand various methods used to estimate the PMI.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how evaluating the viability of the PMI estimation formulae as they are applied in Louisiana will benefit future PMI estimations in that region.

Forensic anthropologists are often called upon to provide an estimate of the period of time from death until discovery of human remains (i.e., the PMI). PMI estimates are often based on the condition of remains upon discovery (i.e., the observed stage of decomposition); however, since many factors affect the pattern and progression of taphonomic processes, interpretation of the PMI can prove to be difficult. Various formulae have been developed that allow quantitative assessment of PMI; two examples of such formulae are the Total Body Score (TBS) method and the Universal Post-Mortem Interval Formula.<sup>1,2</sup> The process of each method entails associating the stage of decomposition of an individual with a numerical score. For the TBS method, a body was separated into three regions (head and neck, torso, and limbs) that are scored individually, then summed before being entered into an equation.<sup>1</sup> The Universal Post-Mortem Interval Formula requires a numerical decomposition score for the entire body, which is also entered into an equation.<sup>2</sup> Ultimately, an output of PMI in days can be obtained from each formula. Theoretically, formulae of this type would provide methods of PMI estimation that were both standardized and statistically sound.

The goal of the present study was to evaluate the application of these two methods in Louisiana. Both formulae were applied on past casework spanning the years 2006 to 2017 from the Louisiana State University Forensic Anthropology and Computer Enhancement Services (LSU FACES) Laboratory. These analyses were performed in concert with the assessment of qualitative PMI estimation in past casework from Louisiana. Using cases with known PMIs, it was determined how frequently an actual PMI fell within the range of the provided estimate range. Both the quantitative and qualitative methods were evaluated in order to determine the best approach to estimating PMI in Louisiana in the future. Only individuals with both known PMIs and available photographs were considered for the study, and the resulting sample size was  $n=79$ . When testing each formula, cases were excluded based on the requirements of each method. Ultimately, the sample sizes were  $n=49$  for the TBS method and  $n=16$  for the Universal Post-Mortem Interval Formula.<sup>1,2</sup> The formulae had varying degrees of success based on the strength of the correlations. When applying the Universal Post-Mortem Interval Formula, statistically significant results were not observed using Spearman's rho ( $p=0.384$  [ $p<0.1214$ ;  $\alpha=0.05$ ]).<sup>2</sup> However, the TBS method produced a strong correlation ( $p=0.7529$  [ $p<0.001$ ;  $\alpha=0.05$ ]) when retrospectively applied to the casework.<sup>1</sup> When considering the qualitative PMI estimation methods applied at the LSU FACES Lab, the actual PMIs of 69/79 individuals (87%) fell within the range of the PMI estimate provided by the anthropologists. While the TBS method may have the potential for future use in forensic casework, qualitative PMI assessment has also been successful in Louisiana.<sup>1</sup>

### Reference(s):

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### Postmortem Interval, Decomposition, Taphonomy



## A140 Utilizing Metabolomics Toward Time-Dependent Metabolite Monitoring in Different Postmortem Specimens During Human Decomposition

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how metabolomics of postmortem specimens provide additional knowledge in comparing and contrasting human decomposition processes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by revealing novel aspects of postmortem metabolomics addressing intra- and inter-human metabolic differences in maggots, decomposition fluid, and soil in proximity to human donors across the early and late stages of decomposition.

The ultimate goal of this ongoing study is to reveal drug impacts on human decomposition and the Postmortem Interval (PMI). This presentation examines how metabolomic analyses provide insights into the highly dynamic postmortem biochemical environment. As small-molecule intermediates of human metabolism, metabolites have a multitude of functions, not only in the living human body, but also after death; however, the complexity of factors affecting human decomposition opens up a large, mostly still-unknown area yielding continuous new questions about postmortem metabolic patterns, including molecular signatures or biomarker discovery.

This research is conducted on human donors, obtained through the Body Donation Program of the Forensic Anthropology Center (FAC), in different microenvironments around the three-acre outdoor laboratory known as the Anthropology Research Facility (ARF) in Knoxville, TN. Samples of soil, maggots, and body fluid were obtained and analyzed for metabolites. Soil (10g) obtained from within the Cadaver Decomposition Island (CDI) from ten enrolled donors and control soil (10g), located at least 1m from the donor, were homogenized, flash frozen, and stored in liquid nitrogen until subsequent metabolomic profiling. Decomposition fluid from the body was collected at various anatomical positions around the body. Hourly records of temperature and relative humidity allowed for the calculation of Accumulated Degree Hours (ADH), a metric that combines temperature and time, to determine specific sampling points for soil and decomposition fluid. Maggots were collected at each instar as well as while migrating from the body. Termination of sampling occurred after the body completed active decay as determined by cessation of secretion of body fluid from the trunk.

Metabolites were extracted from 70mg–80mg soil, 30–50mg maggots, and 70–80mg decomposition fluid using a procedure adapted from Lu et al.<sup>1</sup> Analyses were performed by Ultra High-Performance Liquid Chromatography-High-Resolution Mass Spectrometry (UHPLC-HRMS) operating in negative Electrospray Ionization (ESI) with a 2.5 micron reverse-phase Hydro-RP 100, 100mm x 2.00mm Phenomenex® Liquid Chromatography (LC) column. The Exactive™ Plus Orbitrap™ Mass Spectrometer was run in full scan mode with a scan window from 80–1,200m/z.<sup>1</sup> A flow rate of 0.2mL/min was maintained throughout the analysis. On average, 90–120 identified and 1,000s of unidentified metabolites were manually selected based on mass accuracy ( $\pm$  5ppm mass tolerance) and retention times ( $\leq$  2min) using an in-house generated metabolite database.

Donor-, location-, and time-related differences in metabolite signatures were statistically evaluated via Partial Least Squares Discriminant Analyses (PLSDA). Initial results indicate that not only does every cadaver show a unique metabolic signature, but also that each CDI soil is metabolically different from the control soil. Each donor had an individual, time-dependent metabolome based on every sampled ADH throughout the monitored decomposition period. Analyzed maggots and decomposition fluid samples show similar donor- and time-dependent trends, whereas body location seems to impact metabolic patterns.

The current results provide an initial impression of the complexity of the temporal metabolic changes occurring after death in different matrices. Ongoing analyses focus on the identification of possible biochemical biomarkers for decomposition. It is anticipated that with further progression of this study and future toxicological analyses, this study will be able to relate postmortem metabolite signatures to certain peri-mortem drug treatments and answer questions concerning drug-induced decomposition alterations.

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### Forensic Chemistry, Postmortem Metabolomics, Biomarker Discovery





## A141 Bacterial Community Succession: Postmortem Interval (PMI) Estimation of Forensic Anthropological Remains

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**Learning Overview:** After attending this presentation, attendees will understand the need for uniform data collection and sampling protocols regarding the postmortem microbiome for forensic anthropological cases, the composition of the postmortem microbiome of these cases, and whether the genera *Bacteroides*, *Lactobacillus*, and *Bifidobacterium* are useful groups for estimating PMI.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing standard protocols and best-practice guidelines for data collection and sampling the postmortem microbiome and demonstrating how these protocols can improve future research endeavors and collaboration. In addition, this presentation will identify potential trends of bacterial community composition in sample groups not previously studied.

Decomposing remains are an evolving ecosystem experiencing ecological succession as biological species colonize the remains and modify the biochemical characteristics and resources available. Relative abundance and composition of bacterial communities respond to these environmental shifts, resulting in a succession pattern that has been found to correlate temporally with decomposition. Once refined, these patterns may be useful predictors for the PMI. There is a relative paucity of studies, however, that utilize data from actual forensic cases involving human remains. In addition, existing datasets are based on disparate sample groups, taxonomic levels, and sampling locations. In this study, human remains and the corresponding substrates were sampled from two forensic cases from the Mercyhurst University Forensic Scene Recovery Team (M-FSRT) and one forensic case from the Erie County Coroner's Office. The PMIs ranged from 24 hours to more than five years.

Feasible sampling protocols and best-practice guidelines were outlined for studying bacterial community succession on forensic human remains, both at outdoor scene recoveries and in laboratory settings, for future research endeavors. Two locations on the human remains were dry swabbed: around the oral cavity and around the anus. The corresponding underlying skeletal element was swabbed if no soft tissue was present. For laboratory settings, the substrate sampled was the laboratory table. For outdoor scenes, the soil was considered the substrate and sampled in two locations: at 0m (under the center of the remains) and 5m from that center. Four samples were taken for each sample type and location.

Aerobic culture methods were utilized in order to identify a target group for quantitative Polymerase Chain Reaction (qPCR) analysis. The families Lactobacillaceae, Neisseriaceae, and Enterobacteriaceae were cultured from all human remains. Streptococcaceae were only cultured from human remains with a PMI less than 24 hours. Except for Neisseriaceae, the presence of these bacterial families is consistent with significant groups reported in existing literature. *Lactobacillus* was isolated from all cases and was selected for further analysis. Anaerobic genera *Bacteroides* and *Bifidobacterium* were selected based on reports in existing literature.

Primers identified by Hauther et al. were then used to quantify the three aforementioned genera via real-time qPCR.<sup>1</sup> Bacterial DNA was extracted using the Powersoil<sup>®</sup> DNA Isolation Kit. The concentration of DNA isolated from each sample was determined by Qubit<sup>®</sup> fluorometric quantification. The sample quadruplet with the highest concentration from each sample was chosen for qPCR. DNA samples were diluted to 1:10 or 1:100 based on performance in preliminary testing of the primers. Differences in presence and relative abundances of all three target genera between sample locations and PMI were observed. Due to the limitations of the sample group, any patterns or observations should not be extrapolated.

### Reference(s):

1. Hauther A.H., Cobaugh K.L., Jantz L.M., Sparer T.E., DeBruyn J.M. Estimating Time since Death from Postmortem Human Gut Microbial Communities. *J Forensic Science* 2015; 60(5): 1234-1240.

### Forensic Anthropology, Taphonomy, PMI



### A142 The Effects of Scavenging on a Donor From the Western Carolina University Forensic Osteology Research Station (FOREST)

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**Learning Overview:** After attending this presentation, attendees will better understand scavenging patterns at the Western Carolina University FOREST.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that scavenging of soft tissues can impact the rate of human decomposition, as well as the position/location of the remains.

This project presents a case study that complements the mounting research relating to the effects of scavenging on human decomposition. It is pertinent for medicolegal investigators to understand their local scavenger guilds as well as recognize the patterns and results of scavenging. Scavenging affects the ability to accurately estimate the postmortem interval as well as determine peri-mortem trauma from postmortem damage. This study also supports the need for further longitudinal studies relating to geographically specific scavengers.

The purpose of this project was to see if scavenging activity impacts the estimated Accumulated Degree Days (ADD), which is used by anthropologists to estimate the postmortem interval. The method developed by Megyesi and colleagues was used to produce an estimated ADD, which was then compared to the actual ADD.<sup>1</sup> A motion-activated game camera recorded the scavenging activity affecting a 67-year-old female donor over a ten-day period during the summer of 2019. The donor died of natural causes and presented no trauma at the time of placement. The cameras recorded the dates, times, and temperatures during scavenging events.

Over the ten-day period, the game camera was activated ~5,340 times by four different local scavengers: American crows (*Corvus brachyrhynchos*), black vultures (*Coragyps atratus*), turkey vultures (*Cathartes aura*), and North American opossums (*Didelphis virginiana*). The game photos were stitched together into a time-lapse video and reviewed to observe any trends in scavenging activity, as well as the overall impact that the activity had on the remains.

Initial scavenging by American crows occurred within two hours of donor placement. Early on, a pattern of scavenging was consistent over the observation period, with crows and vultures scavenging during the day and opossums at night. By the second day, the cranium and sections of the limbs showed signs of partial skeletonization. This resulted in a high estimated ADD ( $737.9 \pm 388.16$ ) when compared to the actual ADD (36.41), demonstrating the influence that scavenging had on the decomposition process of this individual. Not only were scavengers removing soft tissue directly from the skeletal remains, they were creating openings in the soft tissues that allowed for greater insect access and activity. By the end of ten days, the donor was mostly skeletonized and had been rotated (~45 degrees) and moved approximately one meter from its original location. Again, the estimated ADD ( $4,073.8 \pm 388.16$ ) was much higher than the actual ADD (187). None of this scavenging activity left any evidence on the skeletal remains.

Overall, two important findings were clearly documented in this footage, that: (1) scavenging increased the rate of decomposition, and (2) scavengers altered the placement of the body from its original depositional orientation and location. This project demonstrates the necessity of long-term studies documenting the effects of scavenging on human decomposition as well as revised ADD methods that account for scavenging activity.

#### Reference(s):

- <sup>1</sup> M.S. Megyesi, S.P. Nawrocki, N.H. Haskell. Using accumulated degree days to estimate the postmortem interval from decomposed human remains. *J. Forensic Sci.* 50 (2005) 618–626.

#### Forensic Anthropology, Scavenging, Postmortem Interval

## A143 Coyote Pup Scavenging as Distinct From Adult Behavior: The Potential for Reproductive Patterns to Inform the Estimation of Postmortem Interval

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**Learning Overview:** After attending this presentation, attendees will understand juvenile coyote scavengers as behaviorally distinct from adults.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing novel insight into variation in intraspecies behavior and its potential impact on the forensic analysis of human remains.

Coyote (*Canis latrans*) scavenging has received considerable attention in taphonomy and ecology research.<sup>1-3</sup> Scavenging coyotes disrupt outdoor death scenes by consuming and scattering human remains, interfering with search and recovery missions, and reducing the probative potential of osteological analysis and the estimation of Postmortem Interval (PMI). Absent from current coyote scavenger data is consideration of the behavior and pattern of juvenile coyote pup scavenging. Juvenile coyotes present differences in dentition, bite force, body size, musculature, and experience. These variables have the potential to impact the rate, pattern, and presentation of scavenging and impact subsequent analyses of human remains.

The impact of coyote pup scavenging was investigated at Colorado Mesa University's Forensic Investigation Research Station high-altitude satellite facility (FIRS-TB40). FIRS-TB40 is located in the Rocky Mountain Region of Colorado at 9,500 feet Above Mean Sea Level (AMSL). The environment is characterized by steep slopes, alpine forests, open moraine grasslands, dramatic weather shifts, high precipitation, high Ultraviolet (UV) exposure, and heavy annual snowfall. Distinct environments foster distinct adaptive radiations that are expected to have a polyvalent impact on behavior, including scavenging. Baigent et al. described the succession and progression of scavenger guilds among a porcine cohort placed at FIRS-TB40 in the summer of 2018.<sup>4</sup> This study demonstrated cooperative and competitive behavior between canid and avian groups and suggested that adult coyote scavenging at high-altitude diverged appreciably from published norms. A higher resolution model of coyote scavenging is reported with an emphasis placed on juvenile coyotes as progenitors of distinct intragroup scavenger behavior. Four pigs (two exposed/two caged) were placed in the summer of 2018 in a longitudinal transect across a 45° slope, each 30 meters apart. Each sample was monitored by a game camera programmed to collect both time lapse photographs and respond to motion in the environment. Carrion were visited three times weekly for a period of eight weeks, photographed, and decomposition documented using the total body score model.<sup>5</sup> At the terminus of active decomposition, carrion were visited biweekly for six-months, photographed, and game camera data collected and analyzed.

Adult coyotes were the primary agents of tissue removal throughout early decomposition. Adult scavenging was characterized by independent, short interval "eat and retreat" behavior, the movement of osseous tissue upslope, and exclusively nocturnal. Adults made no attempt to disrupt the caged carrion or the centrally located exposed carrion. Juvenile coyotes appeared six-weeks postmortem (consistent with seasonal patterns of gestation and weaning), scavenged nightly across a 45-day window, and diverged from adult behavior significantly. Divergence is categorized as: (1) novel behavior (juveniles were cathemeral and worked individually, or in packs of two to four individuals); (2) tenacity (juveniles were observed digging under and burrowing through cage panels); (3) longer feeding periods (juveniles were observed in scavenging phases that averaged five hours in length); and (4) exploitation of novel resources (juveniles exploited carrion previously ignored by adults). Additionally, unlike adults, juveniles were not dissuaded by desiccated tissue; tissue removal was characterized by longitudinal peeling and did not include skeletal involvement. Juveniles were intermittently observed scavenging under the supervision of one to two adults. Adults did not feed during these phases, suggesting that scavenging presents the opportunity for intergenerational training and that juvenile feeding is prioritized.

Juvenile scavenger patterns are critical to understanding intraspecies variation in behavior that may impact scavenger drive, and the presentation and analysis of postmortem defects. Because coyote reproductive cycles are consistent and predictable, the identification of juvenile scavenging has the potential to be temporally co-related and inform the estimation of PMI. Future study will focus on higher resolution descriptive models of the tissue change associated with juvenile scavenging, as well as impact on patterns of skeletal dispersal in the post-deposition environment.

### References:

1. J. Ward et al. Home range size, vegetation density, and season influences prey use by coyotes (*Canis latrans*). *PLoS ONE* 2018; 13(10): e0203703.
2. Haglund W.D., Reay D.T., Swindler D.R. Canid scavenging/disarticulation sequence of human remains in the Pacific northwest. *J Forensic Sci* 1989;34(3):587-606.
3. Haglund, W.D. Dogs and Coyotes: Postmortem Involvement with Human Remains. In Haglund W.D., Sorg M.H., eds. *Forensic Taphonomy: The Postmortem Fate of Human Remains*. NY: CRC Press, 1997; 367-82.
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### High Altitude, Coyote Scavenging, Human Decomposition



## A144 The Patterns of Striped Skunk Scavenging on Human Remains

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**Learning Overview:** After attending this presentation, attendees will better understand the patterns of striped skunk scavenging on human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing examples of striped skunk scavenging to assist in distinguishing postmortem scavenging from peri-mortem trauma that may be associated with cause of death.

Striped skunks (*Mephitis mephitis*) are common throughout most of North America. They are nocturnal and generally solitary, often living in burrows created by other animals. Striped skunks are omnivores, predominately subsisting on small vertebrates, insects, eggs, and plant material.<sup>1</sup> Scavenging is a part of their regular diet. However, cases of skunks scavenging human remains are rarely documented. Existing documentation provides a limited description of observed scavenging.

Seven cases of striped skunk scavenging were observed at the Forensic Investigation Research Station (FIRS) in Whitewater, CO, between November 2017 and June 2019. At the FIRS, human remains are laid outside to study decomposition. The outdoor facility is fenced to allow access to small scavengers while excluding larger scavengers. The scavengers observed include skunks, feral cats, mice, and birds. When a body shows evidence of scavenging, donors are documented daily and monitored with game cameras.

Skunk scavenging usually started before the onset of moist decomposition and continued well into moist decomposition. In one case, scavenging continued into mummification. Scavenging was done at night. The duration of scavenging was 29–105 nights with active scavenging 12–48 of those nights. In two cases, the donor was only scavenged once. Skunks did not show preference for any specific donor beyond state of decomposition. Scavenging often occurred on multiple donors on the same night.

Six of the cases occurred in the winter. Skunks were present for all but 12 nights in November and December 2018. Over the two years covered in this study, five of seven donors placed October–December were scavenged by skunks (two in 2017, three in 2018). In cases in which skunks scavenged bodies more than once, the scavenging duration was longer in winter than in spring.

Initial stages of skunk scavenging were often marked by bone exposure in the limbs, primarily the arms, followed by the legs. While bone was exposed in five of the seven cases, only one of those cases showed macroscopic damage to the bone. In this case, the distal radius and ulna were broken, likely in the process of removing the hand. This was the only evidence of disarticulation in any of the skunk scavenging cases. In three cases, skunks preferentially consumed subdermal tissue. Game camera images show a skunk holding the skin back with its paw as it consumed the underlying tissue. This pattern of tissue consumption gave a hollow appearance to the impacted tissue.

In one case, irregular defects, puncture marks, and parallel linear striae were present peripheral to the scavenged areas. This appeared to be related to efforts by the skunk to reposition the limb or climb on top of the donor while scavenging. Skunks were seen climbing on top of the donor in nearly every case, but this single case was the only case where the activity impacted tissue.

Patterns of skunk scavenging thus far appear to be distinct from patterns observed in two cases of feral cat (*Felis catus*) scavenging at the FIRS.<sup>2</sup> Notable differences between the two scavengers are the timing of bone exposure, the type of differential tissue consumption, the prevalence and nature of peripheral defects, and seasonality. Both species preferentially scavenged the limbs and generally avoided involvement of bones. While there is there are only a small number of documented cases for each of these species, noting the impact of these scavengers will expand current understanding of local scavenger communities.

Defects caused by scavengers can be confused with or conceal the impact of peri-mortem trauma. Recognizing and understanding the patterns of scavengers will assist investigators in determining source and timing of tissue damage.

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1. Greenwood R.J., Sargent A.B., Pieh J.L., Buhl D.A., Hanson B.A. Foods and foraging of prairie striped skunks during avian nesting season. *Wildlife Society Bulletin* 1999 autumn; 27(3):823-832.
2. Garcia S.N., Smith A.J., Baigent C., Connor M.A. The Scavenging Patterns of Feral Cats on Human Remains in an Outdoor Setting. *Proceedings of the American Academy of Forensic Sciences*, 71<sup>st</sup> Annual Scientific Meeting, Baltimore, MD. 2019.

### Skunk Scavenging, Taphonomy, Postmortem Scavenging



## A145 Instars and Stripes: The Scavenging Behavior and Taphonomic Contribution of the Striped Skunk

Rachel E. Smith, BS\*, Marquette, MI 49855; Jane Wankmiller, PhD, Northern Michigan University, Marquette, MI 49855

**Learning Overview:** After attending this presentation, attendees will understand scavenging activities of the American striped skunk (*Mephitis mephitis*) as they relate to human taphonomy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting observations of the scavenging activities of the striped skunk, which may affect trauma and entomological analyses associated with medicolegal investigations.

Scavenging frequently plays a role in the taphonomy of human remains in outdoor contexts. Certain scavengers are known to scatter remains, while others affect a case simply by consuming tissues, which can affect the decomposition sequence and potentially the determination of the postmortem interval. Understanding the type and behavior of scavengers is thus a crucial part of analyzing remains found outdoors.

The research in this presentation was conducted at the Northern Michigan University Forensic Research Outdoor Station (FROST) in Marquette, MI. Common scavengers and carrion feeders of this area include coyotes, bears, gray and red foxes, eagles, gulls, and turkey vultures, though only red foxes and skunks have been observed during data collection activities at FROST. To prevent large scavengers and carrion birds from harming or removing the donor remains at FROST, each body is protected by a large metal mesh cage.

FROST is unique in its location as a forensic research facility, experiencing long, often extremely cold, winters with substantial snow accumulation and several freeze-thaw cycles in a given year. This climatic characteristic markedly affects the decomposition process, but may also affect the scavenging behavior of animals in the area. To better understand the scavenger activity as well as non-scavengers (e.g., songbirds, rodents, deer, etc.), FROST researchers set up trail cameras near recently placed donor remains. The cameras are checked for functionality, and the images are downloaded on a regular basis. The cameras are currently programmed to capture four-image bursts each time they detect motion, 24 hours per day. On several occasions, scavenger activity was noticed on donor remains prior to camera placement, resulting in the setup of a camera in a location that may not have been of interest initially.

Based on previous sightings of foxes and other small mammals in and around the site, many of the animals captured on the trail cameras were unremarkable (e.g., foxes, rabbits, squirrels, and birds), and none have been observed penetrating the cages and scavenging the donor remains. The striped skunk, however, was somewhat surprising, as it represents the overwhelming majority of documented scavenger activity at the site and has repeatedly managed to get inside the protective cages. The skunk appears to be the only recently active scavenger, and trail camera photos demonstrate the consumption of both human tissue and maggot masses when a skunk is present.

Through analysis of the trail camera images and daily observations of the donor remains, FROST researchers have noted two distinct feeding times, as a skunk is often recorded in the late evening and then again in the very early morning, with generally less activity in-between. Skunks also appear to have a preference for scavenging the tissue of the limbs and face, similar to documented observations of raccoons, although there is footage of one skunk consuming tissue from the abdomen of a donor.<sup>1,2</sup> Scavenging of maggot masses is most commonly evidenced by clean-scraped dirt in areas where maggot masses have been observed, indicating that the skunks have scraped up and consumed the maggots. For several months, FROST was frequented by what appeared to be a single skunk, but since mid-July of 2019, four new skunks have been documented. These additional skunks are smaller than the original, suggesting a sharing of knowledge regarding a reliable food source from parent to offspring.

While skunks are known to be carrion feeders and have been observed by researchers during previous taphonomy studies, this is the first instance, per research, of skunks not only representing the primary mammalian scavengers at a site, but also apparently displaying behavior indicating a transfer of knowledge of the human tissue as a food source.<sup>3-5</sup> These observations contribute to the understanding of the ways in which the scavenging activities of the American striped skunk affect human taphonomy.

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1. Steadman D.W., Dautartas A.M., Mundorff A.Z., Vidoli G.M., and Jantz L.M. Differential Raccoon Scavenging among Pig, Rabbit, and Human Subjects. *Proceedings of the American Academy of Forensic Sciences*, 68<sup>th</sup> Annual Scientific Meeting, Las Vegas, NV. 2016.
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### Scavenging, Forensic Taphonomy, Skunk



### A146 Out of the Jaws: Identifying Postmortem Alligator (*A. mississippiensis*) Scavenging on the Human Skeleton

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**Learning Overview:** After attending this presentation, attendees will understand how to differentiate postmortem change inflicted by American alligator jaws on human skeletal remains from damage made by other scavengers. Attendees will also understand how to easily exclude intentional trauma implicated by a human being, by way of proper identification of the postmortem change caused by alligators.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing clear examples of scoring, punctures, pits, scalloping, and crushing and explain how access to a faunal skeletal collection and zoology methods helped to identify the species (and size) of the scavenger.

The American alligator (*A. mississippiensis*) is one of the two native species of crocodylians living in Florida. Numbering approximately 1.25 million in total, the largest alligator population persists in southwest Florida within the tropical wetlands of the Florida Everglades.<sup>1</sup> With the ability to reach lengths over 12 feet (3.6 meters) and bite force exceeding 2,000psi, alligators create a problem for medicolegal death investigations.<sup>2</sup>

Existing references provide examples of tooth pits, punctures, scoring, and furrows and elaborate on the mechanics of bone crushing but tend to focus on general terrestrial scavenging with few exceptions.<sup>2,3</sup> This study contributes by providing photographic and metric examples of alligator damage to skeletal remains while also providing methods for estimating alligator size using methods borrowed from zoology and applied to forensic casework. No less important, these findings are presented with the permission of the decedent's family.

This case was evaluated by a consulting forensic anthropologist for the medical examiner's office, who was called upon to assist with the death investigation. Time since death analysis revealed the decedent was in an aqueous environment for less than two weeks. Approximately 50% of the skeleton was recovered, and 25% presented with postmortem carnivore scavenging damage. This study crossed interdisciplinary borders by delving into the zoology literature, finding methods for estimating overall size of an alligator based on cranial and dental measurements.<sup>4</sup> Using a comparative specimen from the Human Identity and Trauma Analysis (HITA) Nonhuman Skeletal Collection, this study estimated the length of the specimen from the tip of the snout to the tip of the tail (10ft). Then, the maximum tooth diameters of the HITA alligator were measured. The tooth diameters from the HITA specimen (maximum diameter ranged from 4mm to 14mm) were compared to the pits and punctures (maximum diameter ranged from 3mm to 10mm) on the remains. This comparison of tooth and defect diameters, with published alligator length estimation equations, allowed for the estimation of the size of the scavenger. In addition, comparison to the known specimen assisted in ruling out the possibility that any of the trauma that was evaluated was caused by intentional human force and rather during the postmortem interval.

When remains are found in water for an unknown period of time, several lines of evidence can assist in investigating how the death came to be, but can easily be lost or disappear through interference by carnivorous scavengers, as well as other environmental factors. Results of the methods presented above are twofold: (1) to increase the ability of various investigating agencies in determining the sequence of events leading to the victim's death; and (2) to assist these agencies in bridging the communication gap between them and the family of the decedent. In doing so, the goals of finding justice for the victim and effectively communicating to ensure peace of mind to their surviving loved ones are met.

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3. Stock, Michala K., Allysha P. Winburn, and George H. Burgess. Skeletal indicators of shark feeding on human remains: evidence from Florida forensic anthropology cases. *Journal of Forensic Sciences* 62, no. 6 (2017): 1647-1654.
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#### Forensic Anthropology, Taphonomy, Carnivore Scavenging



## A147 Does Donor Skin Color Affect Total Body Scores (TBSs)?

Crystal L. Crabb, BS\*, New Braunfels, TX 78130; Megan F. Veltri, BS\*, Texas State University-San Marcos, San Marcos, TX 78666

**Learning Overview:** After attending this presentation, attendees will understand how the discoloration categories used by Megyesi et al. are population specific, making early stage decomposition challenging to estimate in American Black, American Hispanic, and Native American individuals.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the impact of skin tone on the assessment of the TBS in early decomposition and, therefore, the estimation of the Postmortem Interval (PMI) on People Of Color (POC).

Since its publication, Megyesi et al.'s method of using accumulated degree days and TBS has been widely used by anthropologists studying human decomposition and estimating the PMI.<sup>1</sup> Megyesi et al. advised that this research was preliminary, citing differential environments and temperature as the most important factors for future PMI research.<sup>1</sup> While the ancestral distribution of the Megyesi et al. sample is unknown, many of the coloration terms are specific to White individuals.<sup>1</sup> The purpose of this research is to ascertain if Megyesi et al.'s fresh and early stage discoloration terms, specifically the pink-white and gray to green categories, play a role in accurately estimating the TBS of individuals with darker skin tones.<sup>1</sup>

This study utilized decomposition photographs for 23 POC (9 American Black, 12 American Hispanic, 2 Native American) and 21 American White individuals. The use of photographs to assess TBS has been previously validated.<sup>2,3</sup> Each photograph was scored using the Megyesi et al. scores for the head and neck, torso, and limbs.<sup>1</sup> Discoloration, such as pink-white or gray to green, were included only in the early decomposition stages, and were the focus of this research. However, donor TBS were recorded until the end of the early decomposition stage, marked by post-bloating of the trunk. Deviations from the discoloration, outlined in Megyesi et al., were noted.<sup>1</sup> Additionally, discoloration had to occur on more than half of the body region to be scored as present. Using frequencies, these scores compared White individuals to POC.

In comparing scores for White and POC, several differences were observed. Individuals that were scored fresh (one point) or black-brown (six points) for the head-neck region did not stray from the listed definition. Similarly, points four and five (brown and brown-black/purging of the head-neck) matched the definitions by at least 95% for both POC and White individuals. The categories that strayed the most from scores and definitions were pink-white with skin slip (two points) and gray to green (three points). While 86.7% of White individuals matched the discoloration for the two-point category, only 21.4% of the POC's point scores agreed with the listed definition. Those whose discoloration was scored as gray to green agreed with the definition more than the pink-white discoloration category for both groups (100% for American White, 45.5% for POC). However, POC still did not match either the pink-white or gray to green discoloration definition most of the time they were scored.

A majority of the Megyesi et al. scoring system is inclusive for all individuals.<sup>1</sup> Regardless, the pink-white and gray to green discoloration categories within the early decomposition stage should be altered or based solely on other known and identifiable changes in decomposition. Terms such as "natural skin color" rather than "pink-white" and "color change" rather than "gray to green" may be more appropriate. Utilizing Megyesi et al.'s discoloration terms reduces the effectiveness of the TBS method.<sup>1</sup> As a result, the estimation of the PMI becomes more ambiguous.

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2. Dabbs G.R., Connor M., Bytheway J.A. 2016. Interobserver reliability of the total body score system for quantifying human decomposition. *Journal of Forensic Sciences* 61(2):445-451.
3. Wescott D., Steadman D., Miller N., Sauerwein K., Clemmons C., Gleiber D., McDanel C., Meckel L., Bytheway J. 2018. Validation of the total body score/accumulated degree-day model at three human decomposition facilities. *Forensic Anthropology* 1(3):143-149.

### Total Body Scores, Decomposition, Discoloration



## A148 Preliminary Investigations for Documenting Human Skeletal Remains in Obstructed Wooded Environments

*Morgan J. Ferrell, BA\*, Orlando, FL 32817; John J. Schultz, PhD, University of Central Florida, Orlando, FL 32816; Megan L. McCollum, University of Central Florida, Orlando, FL 32816*

**Learning Overview:** After attending this presentation, attendees will better understand how Close-Range Photogrammetry (CRP) can be used to record forensic scenes in which there are human skeletal remains in obstructed outdoor environments. This presentation will focus on using CRP data collection methods to record a simulated forensic scenario constructed in an oak hammock environment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing preliminary investigations for using CRP to record scattered skeletal remains that are partially obstructed from view in a wooded environment. Building on previous photogrammetry research documenting skeletal scatters, this research will adapt these methods for obstructed wooded scenes.

Mapping and the comprehensive documentation of crime scenes are essential steps in forensic investigations. However, scenes involving human skeletal remains in wooded environments are very challenging to document. By mapping the spatial distribution of all recovered evidence, including skeletal remains, investigators may be able to recreate the scene and infer post-depositional processes. The purpose of this presentation is to demonstrate how CRP can be utilized to preserve contextual information through the creation of 3D models of forensic crime scenes involving human remains in wooded obstructed environments.

Photogrammetry is a field of study concerned with the use of photographs to obtain reliable measurements and the application of software to create 3D models. In particular, the software used in this study implements a Structure-from-Motion (SfM) algorithm to produce 3D models that allow for additional measurements or observations after the scene has been processed. While 3D modeling using CRP is frequently used to record archaeological sites, it has yet to be widely implemented in forensic settings. There are many advantages to using CRP in forensic scenarios, including recording contextual information as a 3D model that is a true-to-scale representation of the scene. Additionally, simply taking only overhead photos may not be viable for scenes in wooded environments, as individual skeletal elements may be obstructed from view. However, for CRP to become widely used for forensic scenes involving human skeletal remains, guidelines need to be further developed to generate the most accurate imagery.

Multiple iterations of a simulated forensic scene consisting of slightly scattered human skeletal material and clothing were constructed in an oak hammock environment. Data collection occurred in the natural lands of the University of Central Florida, with a ground surface consisting mainly of leaves. Remains were scattered around the bases of a few trees so that individual skeletal elements would be purposefully obstructed from view. Photographs were taken freehand using a Nikon® D7200 camera from five view angles while moving around the scene, with additional parallel overhead shots being taken with the camera attached to an extension pole. Close-up photographs of individual bones and joint surfaces were also taken. Cultural Heritage Imaging calibrated photogrammetric scale bars with coded targets were placed around the scene, and 3D models were processed using Agisoft® MetaShape® Professional.

Preliminary results of the models were very encouraging. The most important advantage of this technique was the ability to view all skeletal elements in one overhead orthophoto generated from the model. Further, the 3D models can be manipulated to view various angles and close-ups of the scene. Two tests were used to determine the accuracy of the models: the Root Mean Square Error (RMSE) and a visual assessment. All models had a RMSE of less than one, which is considered an optimal level of error for an accurate model. When viewing the entire model zoomed out, there are very few visual errors. When zoomed in, minor issues can be noted primarily along the bone edges as well as visual distortion along the interface of the bones with the ground surface.

The main limitations affecting model quality were shadows and constantly changing lighting conditions. These shadows, which are caused by surrounding trees and the photographers themselves, are unavoidable unless photographing while overcast. Additionally, the leaf ground surface proved challenging because moving around the scene during data collection occasionally disturbs the ground surface and, therefore, the model quality. While preliminary, this research generated near high-quality models with minor visual errors. However, further research is still needed to produce models with fewer distortions when zoomed in as well as to develop guidelines for documenting larger obstructed scatters.

### Photogrammetry, Scene Documentation, Forensic Archaeology





### A149 Refining Data Collection Parameters for the Photogrammetric Documentation of Outdoor Skeletal Scatter Scenes: Considering Ground Surfaces

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**Learning Overview:** After attending this presentation, attendees will better understand the applications and limitations of documenting outdoor crime scenes using Close-Range Photogrammetry (CRP). This research focuses specifically on the comparative 3D rendering of different ground surfaces in two simulated outdoor crime scenes containing real human skeletal material.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility of CRP and describing how it is advantageous for application in documenting outdoor forensic crime scenes. The effects of different ground surfaces and other environmental factors that affect the 3D documentation process are also discussed.

Forensic archaeologists seek to preserve the context of the entire scene to accurately capture the taphonomic changes and reconstruct events. In archaeology, photogrammetry has been used increasingly to document scenes. However, there has been limited application in the field of forensic science. The purpose of this presentation is to refine CRP methods to use for forensic crime scene reconstructions involving human skeletal material with limited scatter of the bones on two different ground surfaces.

Digital photogrammetry relies on the systematic collection of photographs around a real-world object and computer software utilizing Structure-from-Motion (SfM) algorithms to triangulate 3D coordinates of overlapping points between two or more photographs. This documentation technique allows for the preservation of the entire 3D context and for the virtual manipulation without destroying evidence. Despite these advantages, there has been little research on the applications of CRP to the field of forensic archaeology.

For this research, two simulated crime scenes were constructed using human osteological material and clothing with minimal dispersal of the bones. These scenes were constructed at two sites on the University of Central Florida natural lands. Sites were selected for two different bottom surfaces: a mixed surface dominated by leaves, and a mixed surface dominated by grass. Prior to photography, ground foliage was cleared. Photographs were collected using a Nikon® D7200 camera and a mix of hand-held and extension pole techniques to photograph six different angles around the scene. The first five angles were photographed using the hand-held technique and the sixth angle consisted of overhead shots using the extension pole. These multiple angles have created accurate scenes with better resolution than previous research. Both scenarios were photographed using Cultural Heritage Imaging calibrated photogrammetric scales to create a scaled model. Both models were processed using Agisoft® MetaShape® Professional software. Measures of accuracy used to evaluate the models include the Root Mean Square Error (RMSE) reported by the MetaShape® software as well as visual comparison of the 3D model to the real-world scenario. Visual comparison consists of two evaluations: visual distortion around subject edges, and ground-subject interfacing issues. These are both visual errors that occur in the texture of the model.

This research demonstrates that for spatially limited and less complex scenes, photogrammetry should be integrated into the data collection protocol. Data collection parameters used in this research include both hand-held and extension pole photography techniques, clearing ground vegetation, and a total of six look angles for the models. Using these specifications, both models have final RMSE scores of less than one, indicating that they are models from which accurate measurements can be taken. Overview of the entire 3D model shows high visual and statistical accuracy. Noticeable visual distortion only occurs when the model is zoomed into scenes with highly complex geometry. In this case, the model of the bones on the surface dominated by leaves shows more visual distortion than the surface dominated by grass. Another visual complication present in the model dominated by grass is dappled shadows over the scene that may have influenced the lighting of the resulting model. Overall, CRP provides an accurate model of the positionality and context of skeletal material and should be incorporated as a methodology for the documentation protocol of outdoor forensic crime scenes.

#### Close-Range Photogrammetry, Outdoor Crime Scene, Complex Ground Surface



### A150 Identification of Bone Using an Alternate Light Source (ALS) in Terrestrial Pedestrian Searches

Sara M. Getz, PhD\*, Idaho State University, Pocatello, ID 83209-8005; Christian Petersen, PhD, Idaho State University, Pocatello, ID 83209; Tanyin Watson, Pocatello, ID 83201

**Learning Overview:** After attending this presentation, attendees will understand that conducting an additional nighttime search of an outdoor scene with an ALS is an effective way to find remains that may be missed during typical daylight searches.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing additional evidence that ALS is a valuable addition to the toolkit used in the search for human skeletal remains.

ALS has been shown to be an effective means of detecting bones and teeth in searches of terrestrial outdoor scenes.<sup>1</sup> This presentation describes preliminary results from the first two phases of a three-phase project aimed at testing existing recommendations for using ALS to detect human remains during pedestrian searches. Phase 1 assesses the light and filter combinations possible using the UltraLite™ ALS One, which is a compact and relatively inexpensive ALS kit that includes a four-setting light source (Ultraviolet, 400nm; low and high intensity Blue Merge Technology (BMT™), 450–480nm; Green, 525nm) and three pairs of filter glasses (yellow; amber; red). Dark amber glasses were also evaluated. All combinations were evaluated for use on non-human bone (fresh; sun-bleached; previously buried/soil-stained) and human bone (commercially prepared anatomical specimens; previously buried historic remains).

Specimens were photographed using a digital camera and commercially available lens filters and also subjectively evaluated by three people. High-intensity BMT™ paired with dark amber filter glasses provided the best visualization of fluorescence in bone with organic material present while simultaneously allowing sun-bleached bone, which had limited-to-no fluorescence, to be seen. It was not possible to identically capture what was observed visually using the selected camera filters, however, using the high BMT™, the most comparable camera filter (orange) to the dark amber glasses also produced the best visualizations.<sup>1</sup> These results are consistent with previous success using light in the 450nm range to identify human remains and the manufacturer's recommendation that BMT™ paired with an amber filter is suitable for the detection of most biological evidence.

Phase 2 simulates a scenario in which a day search fails to yield 100% of the remains suspected within a search area. Two test areas of 705 square meters each (23.5 meters by 30 meters) were selected in the semiarid hills of Pocatello, ID, where sagebrush, high desert grasses, and foxtails are the dominant vegetation. Search areas were bounded at one end by a fence marked with flagging tape at 3.5 meter intervals, the other end by reflective markers also placed at 3.5 meter intervals, and on either side by reflectors at 5 meter intervals as guides for bone mapping and search navigation.

A test sample of 35 elements including anatomical, sun-bleached, and stained bones and teeth was distributed in realistic patterns within each area. Remains were partially buried, hidden under sagebrush, obscured with grasses, and inserted into animal burrows with some elements placed in groupings that commonly occur in surface scatters (e.g., mandible and two incisors, two tarsals). Each element was photographed and mapped.

Three-person search teams of student volunteers evaluated each area during the day and at night, for a total of eight searches (four day/night pairs). For the same group of searchers, the average time needed for a day and night search were comparable (Group A: 35, 43 minutes; Group B: 60, 56 minutes, respectively). Between two and ten elements were missed by each group during each day search, however, most were found during each subsequent night search in the same area, resulting in final recovery of 33–35 items (94%–100%) in each search. These preliminary results support integration of ALS into outdoor pedestrian search procedures.

Additional searches are scheduled for Fall 2019 to investigate the effect of increased experience, changing seasonal vegetation, and larger search groups on these results. Phase 3 will evaluate revised search recommendations under more realistic conditions (e.g., no marked search boundaries, mixed search groups of osteologists, law enforcement/medicolegal professionals).

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#### Alternate Light Source, Pedestrian Search, Forensic Anthropology



### A151 A Retrospective Study of Forensic Fire Searches and Recoveries at Mercyhurst University: 1983–2019

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**Learning Overview:** After attending this presentation, attendees will better understand the forensic archaeological, taphonomic, and anthropological approaches to fatal fire scenes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by examining trends in the role of forensic anthropology and archaeology in forensic fire scenes and by providing a set of best practices toward scene processing and human remains recovery for a variety of forensic fire scenes.

A retrospective review of forensic anthropology cases from the Mercyhurst University Forensic Case Data Bank (1983–2019) was performed for fatal fire scene searches and recoveries processed by the Mercyhurst Forensic Scene Recovery Team (M-FSRT). This included fire cases in which a forensic archaeological search and/or recovery was performed, regardless of whether human remains were found.

Data were collected from each fire scene on location (state, county), fire type (structure, vehicle, outdoor, mass disaster, other), seasonality of fire recovery (spring, summer, fall, winter), and manner of fire (accidental vs. intentional). Structure fires were defined as any fire scene that involved burning of all or part of a structure, such as a house or shed. Outdoor fire scenes were defined as any outdoor area where material had been burned, including fire pits. Vehicle fires were defined as any vehicle subjected to fire modification, including fires that occurred as the result of a vehicle crash incident as well as intentional fires to stationary vehicles. Mass disasters were defined as scenes involving a large area and multiple victims, such as plane crashes. Each fire recovery or search was considered one case regardless of the number of victims.

A total of 1,602 forensic anthropology cases conducted between 1983 and 2019 by Mercyhurst University personnel were reviewed. A total of 205 forensic archaeological searches and/or recoveries were conducted over the 37-year period, representing 12.7% of all cases (with 386 total recovered individuals); 36 were categorized as fire searches/recoveries.

From 1983 to 2009, 16% of all searches/recoveries involved fire, while in the past ten years (2010–2019) that number has increased to 18%. More than half of the total fire recovery cases were conducted between 2015 and 2019, with the greatest number of cases occurring in 2017 ( $n=8$ ).

Fire searches/recoveries spanned four states (Nevada, New York, Ohio, and Pennsylvania), with the majority of fire recoveries conducted in Pennsylvania (72.2%). In Pennsylvania, fire recoveries were conducted in 16 of 67 counties, the majority of recoveries from the northwest region of the state. While fire recoveries spanned all seasons, the greatest number of cases occurred in the summer (33.3%), followed by the fall (24.2%).

Of the fire recovery cases included, 36.4% were outdoor fires scenes, 24.2% were structure fires, 18.2% were vehicle fires, and 21.2% were fires as a result of mass disasters. Of the outdoor fire scenes, one case involved a ruptured gas well, and three cases involved burn barrels to either facilitate the burning process or to store burned remains.

In the cases reviewed from Mercyhurst, the majority of fire scene recoveries were related to plane, train, and automobile accidents, accounting for 42.9% of fire scenes. Intentional fires related to the disposal and concealment of a body constituted 34.2% of cases. The manner of fire scene was unknown for 22.9% of cases. The majority of searches/recoveries were conducted in rural areas where large yards and close access to secluded wooded areas allow for prolonged burning, potentially accounting for the large number of intentional fire cases.

Fatal fire scenes present unique challenges to the recovery process. The application of forensic archaeological methods in such cases can be essential to preserving context and association of evidence and human remains. Considering the increasing involvement of forensic anthropologists in fatal fire recoveries and the diversity with which fatal fire scenes may present, it is imperative that forensic anthropologists have sets of best practices for how to approach scenes of this nature. This retrospective review covers a wide variety of fatal fire scenes and archaeological recovery approaches from which recommendations for recovery efforts can be gleaned.

#### Fatal Fires, Archaeology, Forensic Anthropology

## A152 An Evaluation of Mitochondrial DNA Success Rates in a Commingled Assemblage From the Cabanatuan Prisoner of War (POW) Cemetery

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**Learning Overview:** The objective of this presentation is to explore analyses differences in mitochondrial DNA success rates by DNA testing strategy, element type, and taphonomic modifier.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing data to aid in better selection skeletal elements for DNA sampling in forensic contexts, particularly when skeletal material is in poor condition and/or commingling is present. This study also examines how various taphonomic alterations may influence DNA success rates.

The Cabanatuan POW Camp was a World War II POW camp located in the Philippines, associated with 2,763 American casualties. Unidentified individuals were eventually interred in the Manila American Cemetery and Memorial (MACM), and historians estimate there are approximately 990 to 1,006 unresolved Cabanatuan individuals.

The Cabanatuan Project, initiated by the Defense POW/MIA Accounting Agency, is a focused effort to identify these unknowns.<sup>2</sup> The remains are commingled and in poor condition.<sup>1</sup> Although each casket presumably designates a single individual, anthropological and DNA analyses have demonstrated as many as seven individuals represented in single casket.<sup>3</sup>

Due to the poor condition of the Cabanatuan remains, many anthropological analyses are not applicable. In addition, the remains were treated with lye and formalin prior to burial at the MACM. Identification of individuals often hinges on DNA results. Selecting skeletal elements likely to yield DNA is crucial to this endeavor. This study examines DNA results to answer two important questions: (1) What elements are most likely to sequence?; and (2) What effect do taphonomic alterations have on DNA success rate?

The DNA results of 20 individual caskets were analyzed. Every element tested for DNA was recorded, and DNA yield was reported as successful if the Armed Forces Medical Examiner System-Armed Forces DNA Identification Laboratory reported a sequence. The type of DNA testing strategy (Sanger or Next Generation Sequencing [NGS]) used for each element was recorded. The presence of commonly observed taphonomic alterations, which included the presence of vivianite (a type of phosphate crystal) and rust staining, were visually assessed, when possible.

A total of 318 sampled elements (404 DNA tests) were included in this study. There were 121 upper limb elements, 112 lower limb elements, 61 teeth, and 19 cranial elements. The remaining elements included a few vertebrae and foot elements. There were a total of 215 Sanger tests and 189 NGS tests. Some elements were sampled more than once, and some samples, after failing to sequence in Sanger, were subsequently tested with NGS ( $n=86$ ). For the purposes of this study, multiple failures of a specific test from a single element were only counted once.

Independence and goodness of fit frequency tests were used to examine differences between DNA success rates of Sanger and NGS. Overall, the difference between successful Sanger and NGS testing for the entire assemblage was slight: Sanger 41.86% (90/215) vs. NGS 41.80% (79/189). However, there were differences in DNA success rates between Sanger and NGS among body regions. NGS had greater success for teeth at 69.70% (23/33) vs. 51.28% (20/39) for teeth in Sanger ( $p<0.001$ ). Sanger had a higher success rate for lower limb elements (60.27%; 44/73) vs. NGS (43.55%; 27/62) ( $p<0.001$ ). There was not a significant difference between NGS and Sanger modalities for upper limb elements ( $p=0.134$ ).

Taphonomic information was collected for available skeletal elements, excluding teeth ( $n=169$ ). Fifty-nine elements were observed with vivianite crystals. Fifty-seven of these elements were tested in NGS and 9/57 (15.78%) sequenced. Only two elements with vivianite were tested in Sanger and neither sequenced. Rust staining was observed on 111 elements. Forty rust-stained elements were tested in Sanger and 55.00% (22/40) successfully sequenced, while only 30.99% (22/71) tested in NGS were successful ( $p<0.05$ ). Forty elements had both vivianite and rust staining; however, taphonomic signatures were assessed independently.

Overall, the Sanger and NGS success rates varied within this Cabanatuan assemblage based on element type. The presence of vivianite was found to indicate poor DNA success in NGS; however, this may be correlated to other underlying factors, such as prior treatment or the waterlogged casket environment. Future studies may further examine this relationship. This information may be useful to other forensic practitioners making DNA sampling decisions for commingled, treated, or waterlogged remains.

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1. Megyesi M.S. 2018. Resolving Commingling and Past Accounting at Cabanatuan Prison Camp Cemetery. *Proceedings of the American Academy of Forensic Sciences*, 70<sup>th</sup> Annual Scientific Meeting, Seattle, WA. 2018.
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### DNA, Commingling, Taphonomy

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### A153 Standardizing the Use of Grave Markers for Unidentified Migrant Remains in South Texas Cemeteries

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the inconsistent use of grave markers for unidentified migrant remains in South Texas and how the standardized use of grave markers could greatly assist the recovery and identification of remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by promoting best practices for tracking unidentified remains in South Texas cemeteries that will improve future exhumation and investigative efforts and ensure compliance with state laws.

The migrant death toll at the United States-Mexico border is a mass disaster, with the highest numbers of deaths currently in Texas. According to United States Customs and Border Patrol statistics, more than 4,000 people have perished trying to cross the border in Texas since 1998, with more than 1,500 of these deaths occurring since 2012. While the Texas Criminal Code of Procedure (TCCP) mandates that all unidentified remains be investigated, sampled for DNA, and tracked for a minimum of ten years, the degree to which South Texas counties are compliant is inconsistent. Because local authorities often lack the funding or personnel to carry out swift, thorough investigations, the vast majority of unidentified migrants recovered in South Texas are buried in local cemeteries without having been forensically examined or sampled. The way these individuals are buried, including the extent to which their graves and case information are documented, can greatly impact the preservation of evidence and the ability to re-locate burials for future identification.

Exhumation efforts by Operation Identification (OpID) at Texas State University seek to recover the remains of unidentified migrants in order to facilitate identification and repatriation. Having conducted pedestrian survey and large-scale exhumations in partnership with the Forensic Border Coalition across multiple counties in South Texas, OpID has encountered many challenges when locating unidentified migrant burials. Besides incomplete and missing burial records, most graves are unmarked or have markers that are temporary, flimsy, and/or lacking valuable case information. Most burials exhumed by OpID have instead had to be located by other means, such as informant memory recall, Ground Penetrating Radar (GPR) survey, and exploratory excavation, all of which are time- and resource-consuming. Remains that are recovered with grave markers that have dates, locations, funeral homes, or case numbers listed have a much better chance of being identified than those that are buried unmarked.

The present study seeks to examine the use of grave markers related to unidentified migrant remains in three South Texas counties in order to assess the consistency and effectiveness with which grave markers assist investigative efforts in the region. It is the overall goal of the present study to promote the standardized use of permanent grave markers that contain relevant case information that can be easily located and tracked over time.

Grave marker data were collected for all unidentified migrant burials that have been exhumed to date by OpID over the course of four exhumation seasons across three South Texas cemeteries from 2017 through 2019. Grave markers were assessed in each cemetery in terms of their material, legibility, and content of case information. Of the 94 burials exhumed by OpID, only 24 (25.53%) contained grave markers, with only 20 containing case-related information (21.28%). The most common case information listed on grave markers was funeral homes ( $N=11$ ), followed by dates ( $N=9$ ), and death record numbers ( $N=8$ ). While grave markers were found to be present in all three cemeteries, differences in the use of metal or plastic and paper markers and in the types of information they contained were found between the cemeteries. Thus far, three of the four individuals who have been positively identified from these recent exhumation seasons were buried with a grave marker (75%).

It is the position of this presentation that grave markers should become standard practice for unidentified migrant burials in South Texas. Especially in counties that lack access to medical examiners or forensic practitioners, utilizing grave markers is an immediate and relatively inexpensive solution that will greatly aid in the ability to recover and identify remains in the future. Promoting the widespread use of permanent grave markers will also ensure county compliance with state laws regarding the handling of unidentified human remains.

#### Migrant Death, Exhumation, Identification



## A154 Assessing Fluctuating Asymmetry in the Crania and Lower Limb Bones of Two Modern Populations

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of how environmental stress can affect the levels of Fluctuating Asymmetry (FA) in both the cranium and the lower limb bones.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding to the ongoing research being conducted involving variables affecting FA and will lead to a better understanding of human variation.

FA, the main focus of this research, occurs when small deviations from perfect symmetry present themselves under conditions of developmental stress. Understanding how environmental stress can affect FA is important in a forensic setting. Individuals that have been exposed to environmental stress during growth and development have the potential to show greater levels of FA in the skeleton. This could reflect the environment of a missing person's childhood, potentially aiding in identification.

Inadequate nutrition, Socioeconomic Status (SES), and climatic changes have been shown to be linked to environmental stressors, which can disrupt developmental homeostasis.<sup>1,2</sup> Skeletal asymmetry in the cranium and long bones can be an indicator of environmental stress that occurred during growth and development.<sup>3</sup> The purpose of this study is to examine a migrant population and a donated skeletal collection to analyze varying degrees of FA between the cranium and lower limb bones from both men and women with different SES. Few studies have compared the prevalence of FA in the cranium and lower limbs of individuals with documented SES.

For this study, a total of 50 individuals were examined with paired long bone and cranial measurements to assess the level of FA. Twenty-seven of the individuals were from the Texas State University Donated Skeletal Collection (TXSTDSC) and 23 were from Operation Identification (OpID), which was representative of the migrant population in this study. This study had three categories of SES: low, middle, and high. The TXSTDSC was separated into one of the three groups using self-reported SES. The OpID population was assumed to be from a low SES because unauthorized immigrants often have significantly lower SES when compared to non-immigrant residents of the United States.<sup>4</sup> Postcranial measurements were collected on all lower limb bones and 3D landmarks were collected from the cranium using a Microscribe® digitizer and 3Skull.<sup>5</sup>

Postcranial measurements were converted to absolute asymmetry percentages and a Mann-Whitney test was used to compare FA between sexes, while a Kruskal-Wallis test was used to compare SES. The results showed that only FA in the fibula was significant when both sex and SES were compared. For the cranium, landmarks were paired and aligned using a Procrustes fit, followed by a Procrustes Analysis of Variance (ANOVA) in MorphoJ to measure left-right asymmetry. The results of the one-way ANOVA showed that there was a significant difference between groups in both the comparison of SES and sex, showing that OpID had lower FA scores than all the TXSTDSC individuals, with a significant difference between OpID and the upper SES TXSTDSC. The results also showed that females had a significantly higher FA scores than males in the cranium.

This research demonstrates that there is a significant difference in FA scores between a donated skeletal collection and a migrant population and between sexes, but not between SES. The present study also suggests that FA is more commonly seen in the cranium when compared to lower limb bones. FA levels represented in this study reflect differences in SES between migrants and high SES TXSTDSC, but not between migrants and the other SES groups in the TXSTDSC. There was also a lack of significant difference between individuals with different SES in the TXSTDSC, possibly suggesting that these individuals experienced similar patterns of environmental stress during childhood, regardless of their self-reported SES. Out of the three long bones examined, only the fibula shows signs of FA. This may suggest a prevalence of FA in non-weight bearing bones, rather than bones that often respond to biomechanical factors.

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### Fluctuating Asymmetry, Craniometrics, Human Variation



### A155 Migrants, Crime, and Unidentified Bodies: The Wits Human Identification Unit (HIU) Experience

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**Learning Overview:** After attending this presentation, attendees will have gained insight into forensic anthropology in South Africa and some of the problems associated with personal identification of the decedents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the advantages of collaborations when attempting to identify the skeletal remains of unknown decedents and will also showcase possible opportunities to gain hands-on experience in forensic anthropology in South Africa.

South Africa is a developing country and home to many documented and undocumented migrants from all over Africa. Unfortunately, crime and violence are rampant. Recent statistics reported that approximately 11% of all deaths in South Africa are due to non-natural causes. The major causes of these non-natural deaths include assault and transport-related accidents. Although murder is not one of the major causes of non-natural deaths, approximately 52 people are murdered in the country every day. This results in a large number of postmortem examinations and forensic anthropological cases. Regrettably, many of these decedents enter the Forensic Pathology Services (FPS) as unknown individuals, and about one in ten in the Gauteng Province will remain unidentified.

To assist with the skeletal analyses of unknown deceased individuals, the HIU, which forms part of the Human Identification and Variation Research Unit (HIVRU) in the School of Anatomical Sciences at the University of the Witwatersrand was established in 2016. This unit works in close collaboration with the FPS located in Johannesburg and assists with forensic anthropological analyses of all cases from the southern cluster mortuaries in the Gauteng Province, which is the most populous region in the country. The mandate of the HIU is to establish a biological profile that can be used by the South African Police Service (SAPS) in conjunction with information provided by the FPS and the International Committee of the Red Cross (ICRC) to make a positive identification. Trauma analyses are also frequently requested, even when the identification is known. By April 2019, approximately 120 cases have been received by the HIU, of which 89 have been completed.

Attesting to the problems in the system, forensic anthropological case analyses during the past two years have included the analyses of numerous cases dating back nearly ten years. The majority of remains examined represented Black adult males. Nearly all cases had dentition present; however, this is often of little use as most South Africans and migrants cannot afford dental care or have perfect teeth and, as such, have no dental records. Many cases exhibit signs of trauma, often with a combination of both antemortem and peri-mortem trauma, suggesting a high-risk lifestyle of many of the victims. Multiple cases of ballistic trauma, including cases of multiple gunshots, are also encountered. These cases are often found at mine sites and are frequently related to “Zama Zamas,” who are mainly illegal migrant gold miners.

Identification of the dead is a basic human right and, despite the difficulties, forensic anthropologists are making every attempt to contribute. Agreements for closer collaboration between FPS, HIU, and the Victim Identification Centre (VIC) of the SAPS have been put in place to aid in the personal identification of unknown and undocumented individuals. Through this collaboration, forensic anthropological case analyses are not only completed for cases from the southern parts of Gauteng, but also from other provinces, such as the Free State, Eastern Cape, and Northern Cape, that do not have their own forensic anthropological services.

Although the identification process has many difficulties, a few success stories keep forensic anthropologists motivated. To date, six cases have been positively identified. Four of these identifications were made through DNA confirmation and two through the use of secondary identifiers, including clothing and tattoos. There are also three more cases that could hopefully be identified. One case is awaiting DNA analysis and two others are waiting for facial approximation. Painstakingly slowly, the unknown and undocumented are being identified.

#### South Africa, Forensic Anthropology, Personal Identification



### A156 A Multidisciplinary Identification Approach of Armed Conflict Victims Exhumed From La Resurrección Cemetery in Granada, Colombia

*Alexandra Semma Tamayo, MS\*, Villavicencio, Meta 500001, COLOMBIA*

**Learning Overview:** This goal of this presentation is to describe the importance of a multidisciplinary approach in the identification of armed conflict victims exhumed from La Resurrección Cemetery in Colombia that were analyzed by the human identification team of the National Institute of Legal Medicine and Forensic Sciences in the Eastern Region of Colombia.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the effort of the Colombian judicial system and medicolegal system to investigate these complex cases from the following perspectives: (1) the recovery of antemortem and contextual information concerning the unidentified victims buried in La Resurrección Cemetery in Granada, Colombia; (2) the exhumation of the cases by the Technical Investigation Corps (CTI) and analyses of these unidentified victims by the medicolegal system; (3) the multidisciplinary approach of the human identification team of the National Institute of Legal Medicine and Forensic Sciences in the Eastern Region of Colombia using the comparison of the contextual information of the deaths and the new information provided by the analyses performed; and (4) the results up to this point and the upcoming challenges.

The phenomenon of missing people in Colombia has been analyzed from different perspectives throughout the years. The recent official numbers given by the National Center for Historical Memory (Centro Nacional de Memoria Histórica [CNMH]) provides an approximate number of 82,998 missing persons from 1958 to 2017; this period corresponds to the origins and growth of the armed conflict as well as the post-conflict phase. During this period, all actors in the armed conflict performed different types of violence against each other, including torture, disappearance, and multiple incidents of combat between these groups and the Colombian army.

The majority of the investigations around combat casualties, especially the victims from the groups outside the law, were focused on cause and manner of death; the identification of the dead was not the main purpose, leaving hundreds of unidentified victims buried in different cemeteries around the Colombian territory. Since 2010, the State Prosecutor's Office, the Ministry of Internal Affairs, and other organizations in Colombia began a characterization of these cemeteries with the objective of achieving a better understanding of the number of unidentified victims buried as No Name (NN), creating strategies to enable an effective recovery, analysis, and identification of these specific cases.

The continuing forensic effort in Colombia must respond to the high number of cases, the lack of antemortem and contextual information, and the small number of specialized forensic teams, which represents an improvement for the victims and their families.

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#### Forensic Anthropology, Identification, Armed Conflict





### A157 The Detection of Cancerous Lesions in Skeletal Remains Using Visual Methods and Radiographs

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**Learning Overview:** After attending this presentation, attendees will have gained insight into the potential benefits of incorporating medical imaging equipment into standard practice for the detection of potentially individuating lesions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the need for the use of medical imaging equipment in a modern, forensic context to detect the presence of cancer in unknown skeletal remains, contributing to the biological profile and leading to possible identification.

Forensic anthropological literature describes the detection of cancerous lesions on dry bone that originated in soft tissue, later metastasizing to the bone. However, the use of medical imaging equipment to detect these lesions is rarely considered standard practice. Given the mechanisms of cancer metastasis, it is expected that more cancerous lesions would begin inside the bone, thus visible only with the aid of medical imaging equipment. Potentially individuating cancerous lesions may not be detected when employing macroscopic analysis of dry bone. These lesions may be excluded from the biological profile and impede the identification of unknown remains. Rothschild and Rothschild's study analyzed the presence of cancer both macroscopically and radiologically, finding that two-thirds of all cancer cases would be missed with visual inspection alone.<sup>1</sup> However, their study sample may not apply to contemporary, forensic contexts. The objective of this study is to determine if the presence of cancerous lesions in a sample of contemporary skeletons is greater when assessed using radiographs compared to macroscopic examination of dry bone, allowing for more accurate accounts of potentially individuating lesions in unknown remains.

The study sample consisted of 30 individuals with reported cancer from the Bass Donated Skeletal Collection. Elements selected for analysis included: skull, vertebrae, sacrum, clavicles, scapulae, sternum, ribs, humeri, os coxae, and femora. All elements were examined in isolation for the presence of visible lesions following the criteria for differential diagnosis established in the paleopathology literature.<sup>2-4</sup> Each element was also radiographed at the University of Tennessee Student Health Center by a trained radiology technician. Radiographic images were analyzed blindly to ensure no bias in knowing if these individuals exhibited visible lesions. Lesion nature, location, size, and margins were recorded. Following analysis in isolation for each method, the sample was reanalyzed with simultaneous macroscopic and radiographic analysis, with the frequency of lesion presence between the two methods being compared.

Preliminary results indicate that 23% of the sample exhibited lesions only visible on the radiographs; 33% of the sample exhibited lesions macroscopically, but also exhibited additional lesions that were only visible on radiographs. These lesions were primarily lytic in nature, though there were also instances of blastic lesions only visible on radiographs. Of the elements selected, lesions appeared most frequently on the skull, followed by the os coxae. Preliminary results indicate that more than half of lesions in these cases could have been missed if traditional macroscopic analysis alone was employed. This presentation can validate the use of medical imaging equipment in a modern, forensic context to detect the presence of cancer in unknown skeletal remains, contributing to the biological profile and leading to possible identification.

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#### Anthropology, Cancer, Radiographs



### A158 The Characterization of Peri-Mortem Trauma on World War II (WWII) German Pilots

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**Learning Overview:** The goal of this presentation is to better understand peri-mortem trauma linked to aircraft crashes during WWII and the consequences of taphonomic damage linked to pilot equipment and coffins.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating peri-mortem trauma within a specific group: WWII pilots. This study helps improve knowledge on skeletal analyses in military contexts and identify a common pattern of trauma.

During WWII in France, temporary burials were established for soldiers while the national military cemetery was being built. In 2018, a temporary cemetery was discovered in Beauvais (Oise, France) during land-use planning work. This area was near the German military airfield used from June 1940 to August 1944. The cemetery was excavated, and 39 coffins were uncovered. At the end of WWII, all the soldiers should have been transferred to the national military cemetery, but some of these were not.

Among these 39 coffins, 23 contained skeletal remains (ranging from a single fragment to several bones), and 16 presented complete individuals. These soldiers were bomber aviators and most of them were buried in their parachute. According to historical records, they fell and died between 1940 and the summer of 1942. Anthropological analyses of each individual included the biological profile and the analysis of pathological and traumatic conditions macroscopic observations.

Among the 16 complete individuals, 7 are well preserved and yielded a complete biological profile. Nine individuals are poorly preserved, and their biological profile was incomplete (most of the time, stature could not be estimated). Postmortem damage (taphonomic alterations) could have been caused by the sediment, the coffin, and/or the military equipment. Fifty-six percent of individuals were wrapped in their parachute and their cortical bone was severely damaged.

Peri-mortem trauma was observed in 14 soldiers (87.5%). Five (31%) had been autopsied and buried without military clothes or equipment. Blunt force peri-mortem trauma was the only type of trauma observed. The most-to-least impacted regions were the lower limb (60%), followed by the skull (56%), the upper limb (35%), the pelvic girdle (25%) and the spine (19%). The thorax was poorly preserved, and it was too difficult to differentiate between peri- and postmortem damage.

Among the types of fractures observed, butterfly, spiral, and segmental fractures were the most recorded on long bones. On the skull, linear fractures were very frequent, but the edges were smooth in several cases, due to subsequent taphonomic damage.

Twenty-three other coffins revealed some bones (from one to several) and almost all coffins with several bones revealed at least one case of blunt force trauma (i.e., one fragment of a butterfly fracture on the femur).

The fracture pattern was very similar in all individuals and is compatible with an air crash combined with a fall from a significant height. Even if that could be contradictory, it is rare to know the exact cause of death of soldiers during WWI and WWII.

This study presents rare cases of bomber aviators buried in the same place who share the same cause of death. In the future, we could compare these results with other soldiers who died during armed conflicts, especially aviators. This study intends to provide a comparative framework for differential diagnosis and possible axes for future research.

#### Forensic Anthropology, World War II, Blunt Force Trauma

## A159 Photogrammetry Using Multiple Camera Systems of Mass Graves in a Humanitarian Context

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**Learning Overview:** After attending this presentation, attendees will understand the potential applications of Structure from Motion (SfM) photogrammetry in humanitarian forensic work of mass graves as an affordable and time-efficient alternative to current contextual recording methods. This presentation also provides an overview of the performance of multiple types of camera systems.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by expanding on current research of SfM photogrammetry, illustrating the potential of multiple camera systems in two types of mass graves. This research broadens the capabilities of humanitarian forensic teams by exploiting already owned or readily available camera systems and computing devices.

Two of the biggest challenges facing international humanitarian forensic efforts is the cost of keeping abreast with the latest forensic techniques and continuously expanding body of forensic knowledge. Recording the context of excavated mass graves is an essential aspect of the forensic anthropological process. The collected records can be used both in analysis and in court to provide evidence that a crime has occurred and give insight into reconstructing the crime.

Current technologies used for contextual recording and processing of mass graves, such as total station units and laser scanners, can be expensive, bulky, and time intensive. SfM photogrammetry represents an inexpensive and easy-to-use alternative for obtaining easy-to-interpret and precise 3D models of multiple types of mass graves.

For many years, SfM photogrammetry has been embraced by archaeologists as a method of preserving and recording cultural heritage at excavation sites. However, it is only in the past few years that the forensic field has seen research of SfM photogrammetry applied to mass graves. Previous research had limited image collection to one camera system while evaluating other variables.<sup>1-3</sup>

This research examines the abilities of multiple camera systems in image collection of two types of mass graves: a large, mechanically dug grave and a long, narrow, hand-dug trench. The images used in the study were collected from the following five camera setups: iPhone® 7 Plus still images, iPhone® 7 Plus stills taken from videos, Canon® D100 still images, Sony® a7ii stills from videos, and a simulated drone using a Canon® G9X with a tall, tilted tripod. The camera systems ranged in cost and degree of automation.

The images were subsequently imported into Agisoft® Metashape® (SfM software) to create the 3D models. Multiple settings within the software were examined. Some settings were determined to be incapable of creating a viable 3D model that provides further recommendations to the field. This research also presents the current limitations of SfM photogrammetry and identifies areas needing more in-depth research and understanding for general international and *Daubert* evidentiary standards to be met. This research also highlights the necessity of properly recording and understanding the variables and settings used by SfM photogrammetry for reproducibility.

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**Structure From Motion, Forensic Anthropology, Humanitarian Forensic Science**

## A160 Age-At-Death Estimation Using Dental Cementum Increments: Validation and Limitations

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**Learning Overview:** After attending this presentation, attendees will understand the potential benefits, limitations, and possible pitfalls of dental cementum annulations when analyzed for age-at-death estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing knowledge of: (1) the potential applications of cementochronology to forensic investigations; (2) specific limitations of this method; and (3) recommendations for critically evaluating and implementing cementum analysis for forensic use.

Age-at-death estimation using dental cementum relies on the attractive principle of yearly deposits that would provide a direct access to chronological age. Dental cementum increments analysis has become a routine method for terrestrial mammalian species, but despite recurrent statements regarding its superior performance in humans, cementochronology remains an under-used method for assessing age-at-death. Demanding histological procedure is surely not the sole reason for disregarding this technique. Protocol discrepancies and inconsistent results lead forensic anthropologists to remain oriented toward classic techniques, more standardized and defined. Yet, frequently used procedures have long been known to yield inaccurate, imprecise, and biased (especially among the elderly) age estimates.<sup>1</sup> Cementochronology is being proposed to broaden the range of methods available; however, any method applied to forensic casework must be proven valid, reproducible, and limitations need to be acknowledged. This work aims to provide a standardized framework to define the method's performance and limits. Compounding the concerns over the precision and accuracy of estimation is the question of applicability of such a method to dental material with different preservation conditions. An additional objective is to evaluate the impact of taphonomical alterations.

The study sample consists of 200 canines from individuals of known age, sex, postmortem, and inhumation intervals from anatomical and anthropological collections, and of 200 canines from archaeological assemblages. Teeth were prepared and analyzed in a standardized procedure to prevent bias. All cross-sections have been assigned an index corresponding to microstructure readability, and the extent of cementum loss has been evaluated using two histological indexes: one exclusively considering microbial action and the other considering other types of physical and chemical alterations. Precision of age estimation was assessed by studying statistical dispersion of annuli counts and accuracy by examining the absolute and relative errors.

Results establish intra-observer consistency and demonstrate that dental cementum analysis achieves a reliable estimation of age at death. Results also demonstrate that cementochronology, like any other method, is characterized by significant limitations. While sex, postmortem, and inhumation intervals do not significantly influence effectiveness, a negative relationship between chronological age and annuli distinctness was observed, and multiple regression analyses demonstrate that these two parameters impact both precision and accuracy. The global accuracy is -4.5 years, but although accuracy for subjects under 60 years of age is excellent, absolute inaccuracy strongly increases for subjects over 60 years of age. This demonstrates that performance of cementochronology cannot be summarized into a single value and that this technique is a two-tier performance method. Furthermore, on subjects with a long Postmortem Interval (PMI), attention should be paid to taphonomy since it paradoxically either increases imprecision or artificially improves precision by limiting the number of usable cross-sections and reducing count dispersion.

To establish guidelines for forensic anthropologists to interpret estimates, threshold values were extracted from 95% confidence intervals. Values far above or below these ranges ought to trigger practitioner or reader doubt.

A user-friendly prediction table computed from regression models is proposed as a means of estimating age at death based on annuli count. This procedure, which represents a compromise between the strong accuracy among the young and the increasing inaccuracy among the elderly, broadens age ranges commonly published in cementochronology but maximizes the likelihood of making an accurate age-at-death estimation.

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### Age-At-Death, Cementum, Taphonomy



### A161 Craniofacial Anthropometric Analysis Between the Nose and Nasal Aperture Utilizing 3D Computed Tomography (CT) Scanned Images From Korean Subjects

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**Learning Overview:** After attending this presentation, attendees will understand the correlation between the nose and nasal aperture of the human face and skull and its practical use for craniofacial reconstruction/approximation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the data of the nose and nasal aperture sizes and its correlation in the Korean skull and face. Since all the data set has been collected from the contemporary Korean population subjects, it should be valuable to predict the nose morphology of the modern Korean faces from the unidentified skulls. Also, by the comparison of other ethnicity group data sets, it could provide the anthropological features of Korean faces and skulls.

This study sought to investigate the correlation of morphology and location between the nose and nasal aperture employing major craniofacial landmarks on the human skull and face for the application of a Korean craniofacial reconstruction/approximation. For the study, 100 subjects were selected from the Korean male and female corpus aged between 19 and 49 years whose the bodies arrived in the National Forensic Service (NFS) Seoul Institute for a series of autopsies within 48 hours of their expirations. Postmortem Computed Tomography (PMCT) was employed to acquire 3D head images of the subjects. A total of 32 landmarks were placed on the nose and nasal aperture of each 3D facial and skull image from each subject, and 76 measurements derived from the landmarks were measured in 3D image analysis software (Materialise Mimics).

In frontal, the position values of some bony landmarks on the nasal aperture were related to the position values of some other landmarks on the nose vertically to the transverse plane. In profile, also the position values of some bony landmarks were related to the position values of facial tissue landmarks horizontally to the coronal plane. In 35 out of 76 measurements, the mean values of males were higher than females. In pronasale, subnasal, and right and left alar curvature inferiors, the mean values of 40s of the subjects were higher than 20s of the subjects in both sexes. Overall, 26 out of the 76 measurements showed statistically significant correlations between the corresponding landmarks on the nose and nasal aperture. Simple regression equations were produced from the results.

In conclusion, this study may suggest that the nose and nasal aperture are significantly related with each other in terms of their morphology and location in Korean subjects. The prediction guideline produced as a regression formula could be applicable to the craniofacial reconstruction/ approximation from Korean skulls.

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**Craniofacial Reconstruction, Craniofacial Landmarks, Nasal Morphology**



### A162 The Determination and Prevalence of Non-Forensic Specimens in an Unidentified Collection at the Georgia Bureau of Investigation (GBI)

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**Learning Overview:** After attending this presentation, attendees will be exposed to the intricacies of determining the difference between forensic and non-forensic skeletal material and will understand the issues faced by medicolegal professionals before and after such materials have been misclassified.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the potential for non-forensically significant material to become confused as forensically significant material without proper analysis by a forensic anthropologist. This presentation will aid in the identification of non-forensically significant materials by highlighting common taphonomic changes, craniometric markers, and curatorial differences.

Collections of unidentified remains at medical examiner offices and law enforcement agencies are believed to represent either forensic cases that are pending identification or forensic cases that are deemed to be “cold cases.” In 2016, legislation was passed that allowed for the burial of a large collection of unidentified skeletal remains at the GBI. The unidentified collection at the GBI is comprised of 272 individuals dating back from the 1960s through present day. Prior to interment of the remains, it was decided that a thorough examination of every case was necessary to create a robust case file in the event that a lead on identification of a set of remains arises. Non-forensically significant specimens are often misclassified as forensic in nature. In the state of Georgia, 153 counties use the GBI Medical Examiner’s Office to help identify osseous material. Once at the morgue, remains are first reviewed by a forensic pathologist prior to requesting an anthropological consultation. If anthropological analysis was not available at the time, non-forensically significant materials were often classified as “cold cases.” This misclassification results in a loss of time, resources, and funds during the investigative process.

This study examines the prevalence of non-forensically significant remains present in the unidentified collection at the GBI and hypothesizes that a number of skeletal remains within the “cold case” collection are not of forensic significance and thus can be eliminated from further investigation. For this study, each set of remains had a complete anthropological analysis conducted, including construction of a biological profile through both macroscopic observations and metric analysis, and received taphonomic and trauma analyses. Craniometric landmark data were collected using a MicroScribe® G2x digitizer and 3Skull analysis software. Postcranial element measurements were also recorded. Metric measurements were analyzed using FORDISC® 3.0 and were compared against both the Forensic Data Bank and the Howell’s Data Set.

This study finds that approximately one-tenth ( $n=24$ ) of the entire unidentified collection at the GBI represent non-forensically significant cases. These include remains that are taphonomically distinguished as belonging to historic burials, of anatomical teaching specimen quality, trophy skulls, and other black market materials. This high prevalence means that forensic anthropologists, forensic pathologists, and medicolegal investigators should remain vigilant when osseous materials are examined to ensure materials are classified correctly. It is also urged that remains that have not been analyzed previously receive an anthropological analysis to ensure they are actually forensic in nature.

#### **Taphonomy, Misclassification, Craniometrics**



### A163 Drugs From Dry Bone: How Toxicology Can Add to the Biological Profile

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**Learning Overview:** By attending this presentation, attendees can expect to discover that drugs may be extracted from dry bones even after a 15-year postmortem interval. A new method using accelerated solvent extraction was successfully applied to samples extracted from well-preserved cadavers during autopsies (serving as a control sample) and cemeterial skeletons buried for at least 15 years.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that drugs may be found in dry bones, including in skeletal remains after a long postmortem interval. In addition, a new effective and low-cost method will be presented to perform toxicological analysis on dry bone.

The anthropological practice is usually limited to macroscopic examination, microscopic observation, and DNA analysis. Indeed, very little literature has investigated the survival of toxicological substances in skeletal forensic material and none in dry bone with a postmortem interval of more than 15 years. The present research shows for the first time the detectability of toxicological substances in known bone material with a 15-year postmortem interval.

In this presentation, samples were extracted from the cranium (compact bone) and ribs (spongy bone) during ten autopsies to serve as a control sample. Samples were collected twice to be submitted to two protocols: the first was directly dried in the oven at 50°C for 24h after separation from soft tissues, thus preserving traces of blood; in the second protocol, samples were macerated until only the bones remained before being dried in the oven at 50°C for 24h, simulating a dry bone situation. All samples were then processed with Accelerated Solvent Extraction (ASE) and the eluates were analyzed using a Q Exactive™ Orbitrap™ mass spectrometer.

Additionally, bone samples were taken from cemeterial skeletons from the Collezione Antropologica LABANOF (CAL) Milano Cemetery Skeletal Collection for toxicological analysis. This collection is a modern and documented osteological collection consisting of unclaimed skeletal remains from the cemeteries of Milan, in agreement with Italian law. In total, 13 cemeterial skeletons with verified or suspected drug addictions, intoxication, or overdose in their associated documentation were selected for toxicological study. Fragments of approximately one gram of bone were extracted from the cranium and ribs and subjected to ASE. Similarly, eluates were analyzed using a Q Exactive™ Orbitrap™ mass spectrometer.

The substances tested included opiates, benzodiazepine, 3,4-Methylenedioxymethamphetamine (MDMA), cocaine, heroin, and antidepressants. The results obtained from the fresh and cemeterial samples show a positivity for chemical substances in the skeletal matrices, demonstrating the possibility of identifying toxicological substances in dry bone, after a postmortem interval of 15 years. In addition, results exhibit the efficiency of the ASE extraction for the purification of drugs and xenobiotic substances in dry bone with various postmortem intervals.

In conclusion, toxicological substances may be found in dry bone, even after a postmortem interval of 15 years. At the anthropological level, the toxicological analysis of chemical substances in bones will provide specific information for the construction of the biological profile for forensic and archeological cases.

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#### Forensic Anthropology, Forensic Toxicology, Dry Bone

## A164 The Detection of Chemical Weapon Nerve Agents in Bone: An Anthropological Approach to Skeletal Toxicology

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**Learning Overview:** After attending this presentation, attendees will understand skeletal toxicological methods and their prospective role in forensic anthropology casework and human rights investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that chemical weapon exposure can be detected from skeletal remains, potentially expanding the ability to collect evidence from atrocities even after prolonged post-incident intervals, and by disseminating anthropologically informed models and methods that improve interpretation of skeletal toxicology results in general.

Forensic anthropologists aid forensic pathologists' determination of Cause Of Death (COD) from skeletonized remains when death is due to physically traumatic causes but cannot provide biological evidence for chemical CODs. Official COD in these cases is often left undetermined. The lack of reliable toxicological tests for skeletal material represents a significant gap in forensic science, especially considering both the opioid epidemic and recent chemical weapon attacks.

Studies show drugs can be detected from skeletal remains, even after years of decomposition.<sup>1,2</sup> Contrary to popular assumption, compounds are most readily detected following acute exposures (e.g., overdoses) rather than chronic abuse.<sup>3</sup> However, the absence of known incorporation mechanisms has hindered application of study results. A model for the incorporation of xenobiotics of forensic interest into the human skeleton, newly proposed as part of this research, suggests drugs and other analytes enter bone via equilibrium-based exchange between vascular fluids, bone interstitial fluid, and the separate water layer bound to each bone crystal.<sup>4</sup> Although most are poor substitutes for bone minerals and rapidly exchange back into vascular fluids, select compounds may adsorb onto mineral surfaces and/or fully substitute into the lattice structure.

Based on their unique chemical properties, Nerve Agent Metabolites (NAMs) may adsorb in relatively elevated quantities. To assess this possibility, methods for the extraction and semi-quantitative detection of NAMs EMPA, iBuMPA, IMPA, CMPA, and PMPA (corresponding to VX, Russian VX, sarin, cyclosarin, and soman) from bone were developed using liquid chromatography/mass spectrometry. A quadrupole time-of-flight mass analyzer was used to identify and evaluate the effects of endogenous bone compounds, while a triple quadrupole was used to achieve lower Limits Of Detection (LODs) of NAMs. Instruments were operated using reversed-phase chromatography and negative electrospray ionization. Bone samples were prepared using rapid demineralization and carbon-based solid-phase extraction. Fortified human femoral bone was used for validation. There were no interferences from the matrix or standards; however, ionization suppression was extremely high, ranging from -11.0% to -87.6% at low analyte concentrations. Citrate was the main source of suppression but could not be fully removed from the samples due to shared chemical properties with NAMs. Mean extraction efficiency was 86.7%–100.5% for low concentration preparations, with Relative Standard Deviations (RSDs) of 2.46%–5.73%. Due to high suppression, this equates to mean total recoveries of 12.8%–77.9%, with moderate RSD values (<20%). LODs were as follows: EMPA=350pg/g, IMPA=20pg/g, iBuMPA=7.5pg/g, CMPA=10pg/g, PMPA=5pg/g. Despite high suppression, these LODs are notably lower than most published for other biomatrices, facilitating detection of trace levels and potentially increasing tolerance for diagenetic loss.<sup>5-9</sup>

The proposed model of xenobiotic incorporation and the validated method were tested using femora from 12 mini pigs exposed percutaneously (high-dose exposure;  $n=6$ ) or intramuscularly (low-dose exposure;  $n=6$ ) to VX *in vivo*. EMPA was not detected in intramuscularly exposed animals. EMPA was detected in 6/6 trabecular samples and 5/6 cortical samples from percutaneously exposed mini pigs; concentrations were highest in animals that died due to their exposure. This is believed to be the first time *in vivo* nerve agent exposure has been detected from bone. Further, detected concentrations and diaphyseal-to-epiphyseal concentration ratios reflected animal exposure history (e.g., dosage level/route and post-exposure survival duration). The results are limited but promising, indicating NAMs interact with bone as a pharmacokinetic compartment and can be extracted from bone postmortem. Importantly, the results suggest toxicological testing of human skeletal remains from mass graves is warranted, although additional studies are needed to maximize testing efficiency.

Further, this research expands the foundation of skeletal toxicology broadly by providing a model for xenobiotic incorporation into bone and specifically by demonstrating a total mineral dissolution sample preparation method with the potential to increase extraction efficiency and lower LODs for multiple drug classes and other analytes. These advances may promote discussions between forensic toxicologists and forensic anthropologists and the resolution of overdose deaths.

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### Bone Biochemistry, Human Rights, Drug-Related Deaths



## A165 Pore Extractor: A Micro-Computed Tomography (micro-CT) Image Processing Suite for Characterizing 3D Pore Morphometry in Cortical Bone Tissue

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**Learning Overview:** This presentation introduces a novel suite of image processing macros for micro-CT images of bone tissue, for application in ImageJ and CT-Analyser. The goals of this presentation are to: (1) visually demonstrate the completely automated extraction of cortical porosity, a predictor of spontaneous fracture risk; and (2) describe a novel structure-strain model for “normal” pore morphometry under low and high strain.

**Impact on the Forensic Science Community:** An eroded and highly porous cortex is a significant predictor of spontaneous fracture risk. Increased porosity can have age-associated (primary osteoporosis) or pathological/drug-associated (secondary osteoporosis) origins. To this end, this presentation develops a structure-strain model for expected changes in pore morphometry over the lifespan. By visualizing the porosity of bone tissue, forensic scientists can better assess a fracture’s traumatic (e.g., forensic) or spontaneous (e.g., osteoporotic) origin.

On grayscale micro-CT images, it seems simple to automatically extract 3D pore networks by binarizing bone (lighter pixels) and pore space (darker pixels). However, small pores and faint trabecular margins are often excluded by a single global threshold for pixel brightness. Additionally, at high resolution, the endosteal boundary separating cortical and trabecular bone is ambiguous and commonly must be manually drawn on numerous image slices. In highly trabecular bones (e.g., femoral neck), it is particularly challenging to distinguish large “trabecularized” cortical pores from adjacent, similarly sized trabecular spaces. A workflow to overcome these challenges was developed using an age series of the matched right-side femoral neck and midshaft fourth rib, with one male and one female per decade from the 20s to the 90s. Whole cross-sections of each femoral neck and rib measuring 10mm long were scanned in a HeliScan™ micro-CT (6.4097µm voxels, 60kV, 80uA, exposure=0.4s).

The resulting image processing suite includes the following modules: (1) Slicewise Brightness/Contrast Adjustment (ImageJ): for each slice, picks the minimum threshold on the pixel brightness histogram that excludes soft tissue and includes bone tissue; (2) Adaptive Thresholding (CT-Analyser): removes mounting fixtures and image noise while isolating faint pores based on local pixel brightness; (3) Segment Merging (ImageJ): merges separately scanned regions of large cross-sections; (4) Marrow Bounding (CT-Analyser): draws the endosteal boundary by morphologically smoothing trabecular struts and cortical pore inclusions, extracting a filled cortical mask and isolated cortical pores; (5) Cortical/Trabecularized Pore Type Differentiation (ImageJ): separates cortical pores from “trabecularized” pores by comparing minimum diameter to marrow distance; (6) RCA/PI (ImageJ): calculates relative cortical area and parabolic index; and (7) Regional Division (ImageJ): divides each slice of the cortical mask into regional halves (rib) or octants (femoral neck).<sup>1,2</sup>

3D pore morphometry was calculated for each regional sub-division and pore type using CT-Analyser. The femoral neck displays an increasing compressive strain along a superior-to-inferior gradient, while the pleural and cutaneous cortices of the rib may display opposing high and low strain. This analysis found that pore networks appear morphologically optimized to resist local mechanical strain. High strain pores are significantly less densely populated, produce lower percentages of porosity, are less convergent with other systems, and are more longitudinally oriented. Such isolated systems would be less vulnerable to the initiation and propagation of microdamage than the dense, broad, and widely convergent pore network permitted in lower strain regions. Age-associated increases in porosity maintain this structure-strain optimization, excepting: (1) significantly lower pore separation due to pore convergence; and (2) superior-anterior coalescence of porosity in the female femoral neck.

This image processing suite fully automates cortical pore extraction from micro-CT images and can be modified for traditional histological cross-sections. This project also describes pore morphometry in high and low strain regions, aiding forensic scientists in identifying age-associated or pathological presentations of porosity that may signify spontaneous fractures.

*This work was supported by an award from the National Institute of Justice. The opinions expressed are those of the authors and do not necessarily reflect those of the Department of Justice.*

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### Cortical Porosity, Micro-CT, Image Processing



## A166 Resolving Commingling Via Osteometric and Isotopic Data

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**Learning Overview:** The goal of this presentation is to inform attendees of the utility of combined osteometric and isotopic data to resolve commingling.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a framework for incorporating independent lines of evidence into a single cohesive model for predicting correct element matches from commingled assemblages of human remains.

Resolving commingling is an integral part of skeletal analysis, allowing for more complete sets of remains for further analysis. While multiple methods exist for the analysis of commingled assemblages, these methods are employed in isolation, with no obvious or intuitive way to incorporate the results of various methods into a single decision-making criterion. A Bayesian methodology can provide a means for incorporating multiple lines of evidence in one cohesive model. Previous research has shown the utility of such an approach to osteometric reassociation.<sup>1</sup> This study extends a Bayesian approach through the incorporation of limb bone measurements and simulated intra- and inter-individual isotopic data in predicting correct matches from small-scale ( $n=10$ ) commingled assemblages.

To associate elements, simulated bone collagen carbon and nitrogen isotope values ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively) were combined with linear limb measurements. Isotope values were simulated as random draws from a normal distribution based on the inter-individual and population-level distributional properties of collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from a Northeast Asian population.<sup>2,3</sup> Osteometric data came from 883 individuals curated at the Bass Donated Skeletal Collection at the University of Tennessee, Knoxville. These data were applied to the reassociation model described by McCormick, using a commingled assemblage size of 10 individuals and 1,000 simulated commingled assemblages.<sup>1</sup> It was expected that the incorporation of isotopic data would have a significant impact on resolving commingling in non-paired element comparisons (articulating and “other” [non-articulating and non-paired element] comparison types), as these comparisons typically show the lowest correct classification rates from osteometric data alone.<sup>1</sup>

The inclusion of isotopic data increased correct classification across all comparisons (17 comparisons or 17,000 simulated commingled assemblages), for an overall correct classification rate of 92.0%. “Other” element comparisons showed the highest increase in correct classification over limb bone measurements alone, with a correct classification rate of 88.8% using both osteometric and isotopic data as compared to 31.0% for osteometric data alone. Articulating comparisons also showed a dramatic increase in correct classification with the inclusion of isotopic data, with a correct classification rate of 89.9% using both types of data and 43.4% correct classification using only limb bone measurements. Paired elements had the lowest increase in correct classification with the inclusion of isotopic data (97.6% overall; 85.2% osteometric data alone); this is likely due to the already-high correct classification rate of paired elements via limb bone measurements. Overall, isotopic data alone provided a correct classification rate of 84.8%.

Independent lines of evidence (i.e., osteometric and isotopic data) are of obvious importance in forensic science. Such evidence provides convergence and increases confidence in results. This study provides an important early step in objectively combining multiple lines of evidence into a single model, which reduces uncertainty and subjectivity otherwise present when an analyst uses multiple techniques in conjunction to arrive at a decision. These results illustrate the strength of this approach to resolving commingling. When utilizing two independent lines of highly informative evidence, such as with paired element comparisons, this model is both powerful and accurate. Perhaps the most impactful result of this study is the ability to resolve commingling beyond paired elements, an underrepresented aspect of research on the resolution of commingled human remains. These results also confirm the conclusions of Berg et al. as to the value of isotopic data in resolving commingling and highlight the strength of isotope testing in the analysis of human skeletal remains.<sup>2</sup>

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### Commingling, Isotopes, Bayesian Modeling

## A167 Rare is Good, Unique is Better: Testing Maxillary Sinus Morphologies for Human Identification

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**Learning Overview:** After attending the presentation, attendees will understand the potential of using morphological evaluations of maxillary sinuses for identification purposes. The goal of this study is to quantify morphological uniqueness of maxillary sinuses and to assess their morphology for human identification purposes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insights into the suitability of maxillary sinus morphologies for human identification, observed on X-ray images.

In forensic sciences, many studies are concerned with qualitative analyses of frontal sinuses and their application for identification. Approaches using measurements and volumetric assessments show promising results.<sup>1,2</sup> However, in view of prompt working routines in anthropological case work, utilization of maxillary sinus morphologies offers quicker results, as the features frequently appear on dental overview images. Even if dental comparisons cannot be applied due to, for example, mutilation of the remains, maxillary sinus morphologies can be extracted and compared. However, before techniques can be used to identify remains, the applicability of the procedure needs to be verified as a means of antemortem and postmortem comparison.

A total of 600 maxillary sinuses was sampled on radiographic images from nine population samples dating from Roman times to the 20<sup>th</sup> century. To simulate antemortem and postmortem situations, images were processed twice, for a total of 1,200 sinuses. Individuals were over 20 years of age and showed no pathological changes in the maxillofacial area. Image analyses were performed separately for right and left sinuses. Standardized anteroposterior radiographic images were used for evaluations. Sinus morphologies were extracted using the software SHAPE ver. 1.3, applying Elliptic Fourier Analysis.

Using Analysis of Covariance (ANOVA) statistics, morphological uniqueness between samples as well as individuals was evaluated. Analyses show statistically significant differences for both sides of sinus morphologies between all nine populations ( $p < 0.001$ ). Evaluations by individual show the same trend. Additionally, all individuals have been analyzed divided into modern ( $n=6$ ) and historical ( $n=3$ ) samples to exclude statistical skewing relating to potential evolutionary changes. Results show statistically significant differences for right and left sinus morphologies ( $p < 0.001$ ). Only slight differences can be assessed between the historical samples. Although p-values for the ancient samples are slightly higher, no skewing can be concluded ( $p < 0.05$ ).

To examine suitability of maxillary sinus morphologies for comparison testing, the simulated antemortem and postmortem datasets were assessed using one-way ANOVA. The results indicate total conformity between the two datasets and potentially each antemortem and postmortem morphology pair ( $p=1.000$ ). To evaluate degrees of similarity and difference between individuals in the two datasets, neighbor joining clustering was used. Measuring Euclidean distances, the dendrograms for right and left side morphologies illustrate total morphological accordance for each antemortem and postmortem data pair. Furthermore, they verify sinus morphological uniqueness, as variability between each matching antemortem and postmortem pair is evident. To test for inter- and intra-observer reliability, 18% of the radiographs were reassessed with Kappa statistics. Statistical analysis were performed using IBM® SPSS Statistics v.26 and Past.

This research provides highly encouraging outcomes, demonstrating uniqueness of maxillary sinus morphologies in a simulated environment. Future analyses should involve real life antemortem and postmortem data to verify and further the results.

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### Maxillary Sinus, Human Identification, Morphological Evaluation



## A168 A Novel Method to Augment Personal Identification in the Medical Examiner/Coroner Setting

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**Learning Overview:** After attending this presentation, attendees will have been introduced to a new method under development for use in medical examiner and coroner offices to augment personal identification through statistical evaluation of circumstantial evidence. Attendees will be able to assess the potential of this method to improve identification outcomes for individual decedent cases and mass fatality cases when results from DNA and other methods are inconclusive or absent.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing the details of a novel, mathematically based method that can assess the weight of pertinent medicolegal case evidence to quantify the strength of a personal identification.

Medical examiners and coroners in the United States are responsible for the identification of unknown decedents under their jurisdictions. Most unidentified bodies receive a timely identification through fingerprints, dental records, or medical records. Other identifications, such as DNA comparison with a family reference sample, take longer but are still usually successful. However, many decedents remain unidentified for decades when the above-listed methods cannot be used or are inconclusive. The reasons for unsuccessful identifications are not typically due to a lack of investigational effort. The fundamental issue resulting in failure to identify bodies is simply that the arsenal of identification methods is still inadequate. In 2016, the Disaster Victim Identification Subcommittee of the National Institute of Standards and Technology-sponsored Organization of Scientific Area Committees released a Research Need Request for “development of population-level likelihood values for circumstantial evidence to be used in support of human identification.”<sup>1</sup>

A method that quantifies the strength of the evidence collected by investigators (e.g., clothing, geospatial relationships of body location and residence, personal effects), as well as skeletal findings (e.g., demographic profile, anomalies, bony reactions to antemortem trauma or pathological conditions) will be a valuable tool in personal identification. This idea has been posited in the forensic literature but has not yet been operationalized.<sup>2</sup> It is time to develop an instrument that applies statistical weight to the available qualitative evidence, test it, validate it, and apply it to personal identification in death investigations.

A Bayesian model was built to describe a population created from pseudo-data representing a random set of 5,000 medicolegal decedents with certain properties. The goal of the model was to estimate a 95% credible interval from the posterior distribution of the model for the proportion of a population that has a given profile of features. The model was fit using the Bayesian modeling language Stan (<https://mc-stan.org>) and the statistical language R (<https://cran.r-project.org>). The final production version will have a user interface to these programs. The model incorporated six features: sex, age, height, simplified ancestry (European, African, Hispanic, and Asian), presence of a tattoo, and evidence of antemortem fracture. The distributions of height, ancestry, tattoo, and fracture were assumed to be dependent on age and sex, but future testing of the model using real data may result in more relationships between features.

The model generally fit the data well, except for tattoos; a misspecification in the model resulted in a prediction of more tattoos in older people than the population would support. A test of the model on one living individual resulted in a prediction that the proportion of similar individuals in the population was small, between 0.29% and 0.36%. This investigational model forms the framework for an expanded instrument under development that will be tested using data from a sample of 5,000 actual medical examiner cases.

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### Identification, Forensic Anthropology, Forensic Pathology



### A169 **Wartime Misidentifications of United States Casualties by the Central Identification Unit (CIU) in Kokura, Japan**

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**Learning Overview:** After attending this presentation, attendees will better understand how the United States military made identifications of war casualties, and prevented misidentifications, during the Korean War.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by examining the certainty about the historical identification of United States war casualties.

The Korea Return Program of the United States Army's Office of the Quartermaster General (OQMG) ran from March 1951, when the first 50 remains were shipped from Japan to San Francisco, CA, until May 1956, when the last of 848 Unknown remains were interred in the National Memorial Cemetery of the Pacific in Honolulu, HI. Over that period, the remains of 28,729 casualties were recovered, identified, and repatriated. How many, if any, of these identifications were in error?

Remains of all casualties who died in Korea were evacuated to the Central Identification Unit (CIU) in Kokura, Japan, for processing and identification. Until July 1951, all casualties were interred in temporary cemeteries in Korea; from that point on, an increasing number were flown directly to Kokura, and by the end of 1951, no remains were buried before analysis. Temporary cemeteries varied in their procedures; at some, remains who did not have a name association (Unknowns) were fingerprinted and had their teeth charted; at others, minimal information was recorded on the interment paperwork. Specialists at the CIU conducted anthropological and odontological analyses; in addition, fingerprints were collected whenever possible for submission to the Federal Bureau of Investigation (FBI).

Following analysis at the CIU, a board of officers at the American Graves Registration Service (AGRS) Zone Headquarters (Zone HQ) in Kokura assessed whether the remains were a satisfactory match to the casualty whose name was associated. If they approved the identification, a copy of the file was sent to the OQMG Memorial Division in Washington, DC, for approval. The vast majority of the time, this approval was rapid. In a sample of 719 identifications made by Zone HQ, approval took a week or less in 17% of cases, and between one and two weeks in 40%. An additional 21% took 15–30 days, 17% 31–90 days, and 5% greater than 90 days. The longer gaps occurred when Memorial Division wanted additional information to support an identification. In some cases, families were queried for civilian medical or dental records; in others, the CIU was asked to reprocess remains to confirm specific aspects of the biological profile.

In the majority of documented cases, these supporting records and analyses confirmed the identification. In a small group of cases, however, the identification was determined to be in error. What do these cases tell us about how errors occurred at Kokura, and who else might have been misidentified?

The most common source of error found in the records is the remains exchange, where two or more individuals were each initially identified as the other. Sometimes these exchanges occurred when the remains were initially recovered and interred, other times when they were exhumed and transported. Most were recognized prior to the identification of the remains by AGRS Zone HQ, whether due to CIU analyses or FBI fingerprint comparisons. Two temporary cemeteries provide an initial idea of how frequent such exchanges may have been. Taegu #1 contained 790 burials and Miryang 1,691; to date, nine remains exchanges have been documented at the former cemetery, and four at the latter. Of these 13, nine appear to have happened during the initial recovery or interment, and four following exhumation.

In a more significant case at Miryang, a field identification based on identification media with consistent age, height, and dental was overturned by a fingerprint mismatch. One year later, a second casualty lost in the same area was identified using the same fingerprints. The casualty originally associated with the remains has still not been identified. In this case, if decomposition had progressed too far for fingerprints to be taken, it is possible that a misidentification would have been approved.

Further study of these records is necessary to determine how frequently significant errors occurred, and whether any misidentifications were actually approved. But preliminary analysis makes it clear that the review process used by the OQMG served its purpose well, and that the vast majority of errors were successfully caught prior to identification and repatriation.

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#### **Military Casualties, Misidentification, Korean War**



### A170 United States State Laws and Human Skeletal Remains: Are Old Bones Better Protected Than Forensic Cases?

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**Learning Overview:** After attending this presentation, attendees will appreciate that laws across the United States require osteological expertise for handling human skeletal remains found in an archaeological context but rarely for forensic cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how state laws often place jurisdiction for handling forensic skeletal cases in the hands of coroners or medical examiners, who likely have limited experience in human osteology.

The ability to strengthen the field of forensic anthropology by recruiting more students ultimately rests on opportunities for employment. Berryman and Lanfear cite major incidents, such as the September 11 attacks, as the impetus for hiring forensic anthropologists, but it is also noted that these jobs are frequently eliminated or reduced in an economic downturn.<sup>1</sup> Passage of federal law in 1990 generated numerous jobs for human osteologists and influenced the passage of many state-level laws to protect unmarked graves on non-federal lands.<sup>2</sup> The goal of the present study was to review current laws to identify states that recognize osteological expertise as necessary for the recovery and analysis of human skeletal remains. Free online legal databases, such as FindLaw.com, were searched by state for terms including, but not limited to, “skeletal remains,” “anthropologist,” or “unidentified human remains.”

Results indicate that most states have laws protecting archaeological remains. An increasing number of states require a “skeletal analyst” with “a graduate degree in anthropology” to be consulted for both the excavation and scientific study of human remains from an unmarked grave determined to be 100 years old or older. However, only a small number of states, such as Georgia and Louisiana, have laws that recommend consultation with an anthropologist on medicolegal cases with no definition of expertise. Of these, Texas is the only state that requires a forensic anthropologist to hold a doctoral degree. By law in most states, coroners, medical examiners, and law enforcement officers are under no obligation to consult an anthropologist on a case involving human skeletal remains. An ideal situation would be for forensic anthropologists to work with licensed pathologists who understand how this expertise benefits death investigation. Yet in certain states, skeletal cases are handled by individuals with little to no education in human osteology. The discipline of forensic anthropology will be strengthened, and employment opportunities will arise, if modern medicolegal skeletal cases require osteological expertise as do archaeological remains.

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#### State Laws, Skeletal Remains, Expertise



## A171 Understanding Current Levels of Forensic Occupational Stress and Wellness: Building a Foundation for Increased Forensic Science Workforce Resiliency

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the mental health issues that are affecting specialists employed in the forensic sciences. Understanding the diversity of perspectives on how occupational stressors affect forensic professionals, particularly those who often work in mass fatality contexts, will help attendees not only identify issues in their own offices, but also suggest potentially beneficial interventions. This presentation will emphasize the unique challenges that forensic professionals face and start an important, data-driven dialogue regarding their specific needs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing a gap in current studies of wellness in forensic science training and employment using quantitative and qualitative results from a questionnaire approved for dissemination by the New York City Department of Health and Mental Hygiene Institutional Review Board (Protocol #19-060). Currently, there is a significant lack of research that looks to address or understand the demands of working in medical examiner and coroner offices, let alone doing so in the wake of mass fatality incidents. Mass fatality incidents have become undeniably ingratiated into American culture, and thus it is vital to analyze potential sources of occupational stress and vicarious trauma that forensic professionals are exposed to as part of their daily work. Despite the analytical resources that have been directed toward studies of occupational stress, burnout, and vicarious trauma in emergency personnel following a mass fatality incident, current research either does not consider the experiences of the forensic staff at medical examiner and coroner offices, or does not focus on the specific, long-term effects a mass fatality incident has on forensic staff at medical examiner and coroner offices.<sup>1-7</sup>

This study deployed a 23-question survey to the American Academy of Forensic Sciences (AAFS) membership as well as operational staff at the Clark County Office of the Coroner/Medical Examiner (CCOCME) in Las Vegas, NV, who notably were tasked with processing the deadliest mass shooting to date in American history (October 1, 2017). Survey questions were designed to measure general levels of occupational stress and levels of perceived mental health support. Additionally, questions measuring the potential relationship between elevated levels of occupational stress and frequent contact with human remains and/or frequent contact with next of kin of human decedents were included in the survey. Questions obtaining general demographic information were also included to assess any additional trends. All questions included options to expand on answers to include qualitative data where possible. The survey was generated through the New York City Office of Chief Medical Examiner's Survey Monkey account, and IP address collection was disabled to ensure all participants remained anonymous. The survey was open for a total of six weeks, during which results from 99 individuals (40 from CCOCME and 59 from AAFS) were collected and analyzed.

Results suggest that future studies should consider occupational stress and wellness concerns of forensic professionals at higher, ethnographic resolutions. CCOCME respondents overwhelmingly (75%) reported that they felt contact with human remains and next of kin of decedents had altered their daily life outside of work hours, reporting changes in their interaction with friends and family. Notably, 50% of CCOCME staff and 74% of AAFS reported that they felt common mental health issues were not adequately addressed in occupational, academic, or professional training. Respondents of both survey groups reported feeling worn out from work (61% of AAFS, and 65% of CCOCME), highlighting the potential benefit of further developing wellness programming for the forensic science workforce.

In conclusion, this study finds that forensic professionals do report increased stress levels related to their daily work, as well as specific responses to mass fatality incidents. Additional qualitative responses to some questions also revealed the personal and individualized experiences in fatality management.

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### Wellness, Vicarious Trauma, Occupational Stress



### A172 Population Structure, Population Heterogeneity, and Sources of Error in the Forensic Estimation of “Race”

*Heather J.H. Edgar, PhD\*, Albuquerque, NM 87131*

**Learning Overview:** The goals of this presentation are to: (1) summarize the sources of error in race/ancestry estimation; (2) discuss the terminology used to describe such estimates; and (3) describe the underappreciated importance of intra-group variation within socially recognized groups traditionally treated as homogeneous.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a deeper understanding of how evolutionary history and social forces have interacted to structure phenotypic variation in the United States.

Forensic anthropologists in the United States have traditionally tasked themselves with categorizing unknown human skeletal remains into socially recognized biocultural groups that have conventionally been referred to as “races.” Recognizing that this term carries harmful social connotations, scholars and practitioners are increasingly using alternative terms, such as ancestry or biogeographic ancestry. Regardless of the term used, forensic anthropologists use various probabilistic methods to assign individuals to these socially recognized biocultural groups. These probabilities result from at least two factors: (1) histories of population isolation, movement, and mixture occurring over the past 75,000 years; and (2) political and socioeconomic forces that determine how our society emphasizes or de-emphasizes groups. Though both of these factors shift over time, structured genetic and phenotypic variation exists today in the United States, permitting categorization, with attendant error, in social and forensic contexts.

In this presentation, the term “bioaffinity” is used to reference the socially recognized biocultural groups that derive from this structure. This study examines the sources of error present in estimation of bioaffinity generated from human skeletal remains. These sources include measurement error, mismatch between sample constitution and sample description, error associated with geographic and temporal population structure, and mismatch between the population an individual was ascribed to in life and that indicated by their phenotypic or genetic variants. Further, this presentation will reflect on the terminology used to describe bioaffinity, including the meaning forensic anthropologists hope to convey with each choice of terms and the ways non-forensic anthropologists may actually interpret that terminology.

Finally, this presentation will discuss intra-group variation. This variation has been well studied for bioaffinities that are recognized to derive from a mixture of parental populations, such as Hispanic Americans. However, intra-group variation often goes unexamined in other groups, such as African Americans, European Americans, and Native Americans. For example, using dental morphological characteristics, contemporary European Americans from New York can be discriminated from those from Tennessee with 88% accuracy. This structured intra-group variation means that the sample from which the categorizing discriminant function derives may or may not represent the group from which an unknown individual derives; the degree of the error associated with this mismatch is generally unknown. Similar traits and tests discriminate Hispanic Americans from New Mexico or Florida with at most 81% accuracy, yet the presence of this population structure is generally recognized among forensic and other biological anthropologists. There is to date a dearth of forensically relevant research considering effects of population structure on other markers of bioaffinity, such as craniometric variation, in African and European Americans. Failure to quantify this intra-group variation has important implications for the collection of reference samples and may lead to fewer positive identifications of unknown individuals.

#### **Evolution, Ancestry Estimation, Intra-Group Variation**





## A173 What Are We Really Estimating? An Evaluation of What We Commonly Refer to as Ancestry Estimation

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**Learning Overview:** After attending this presentation, attendees will better understand that the population history of the United States allows for providing estimates beyond what is usually considered ancestry estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting that anthropologists can go beyond ancestry estimation when generating biological profiles.

While it has been stated that it is not possible to sort humans into more categories than continental origins, forensic anthropologists do this all the time, often working within a continent.<sup>1</sup> Forensic anthropologists commonly refer to this practice as ancestry estimation. But is ancestry what we are really estimating? Ousley et al. demonstrated that populations can be sorted by social race categories within the United States, by time period, by ethnic affiliation, and by geographic boundaries.<sup>2</sup> Why then is ancestry estimation the most commonly used terminology? The term “ancestry” was used to replace the word “race” to distance forensic anthropological estimations from a typological and racist past. However, the term ancestry refers to ancestral origins, which could include continental origins or ethnic affiliation. Ancestral categories such as European, African, or Asian are very broad and not especially informative for forensic case work. The purpose of this presentation is to address whether forensic anthropologists are estimating ancestry or something more specific, whether or not such estimations are informative, and how forensic anthropologists should refer to these estimations.

To address questions surrounding what is commonly referred to as ancestry estimation, particularly in the United States, craniometric data, following measurement definitions from Howells, from West Africa (Gold Coast and Cameroon: male=82, female=39), Europe (Germany and the Czech Republic: male=152, female=64), and the United States (American Black: male=213, female=175; and American White: male=282, female=193) were utilized in discriminant function analyses, using the custom import function in *FORDISC*® 3.0, with stepwise variable selection.<sup>3,4</sup> The West African and European groups represent the 19<sup>th</sup> century and American Black and American White groups are separated into 19<sup>th</sup>- and 20<sup>th</sup>-century groups. Analyses were run separately for males and females.

The results for the six female groups indicate the overall classification rate is 67.5%. The West African and European groups have the highest classification rates (97% and 73%) while the 19<sup>th</sup>- and 20<sup>th</sup>-Century American Black and White females range from 46%–68%. For the males, the West African and European groups have the highest classification rates (96% and 88%) while the 19<sup>th</sup>- and 20<sup>th</sup>-century American Black and White males range from 57%–65%. For both the males and the females, the plot of the Mahalanobis distances indicates that the 19<sup>th</sup>- and 20<sup>th</sup>-century American Black groups are intermediate between the European and American White groups and the West African group. The West African group is more dissimilar to the American Black groups, while the European group shows significantly more overlap with the American White groups, for both males and females. When 19<sup>th</sup>- and 20<sup>th</sup>-century American Blacks and American Whites are run separately, classification rates increased (up to 96%), suggesting that morphological differences exist in both groups that provide good classification rates.

The overall discriminant functions suggest that West Africans and Europeans can be differentiated from American Whites and Blacks with high classification rates. While American Blacks and Whites have ancestral origins in West Africa and Europe, they are also uniquely American and both can be assigned group membership to American subpopulations. The population history of the United States includes (but is not limited to) the migration of Europeans and the forced migration of enslaved Africans to the New World, resulting in admixture that shaped population structure. Correspondingly, miscegenation laws, institutional racism, and assortative mating practices have also shaped population structure in the United States to the degree that it is possible to estimate ancestral origins in addition to group membership suggestive of both present-day geography and population substructure. Therefore, an estimation is dependent upon the question asked, and the terminology used is largely context driven. What forensic anthropologists are, or should be, doing is estimating population affinity, a more specific and useful estimation than the more general estimation of ancestry.

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### Ancestry, Biological Affinity, Estimation

## A174 The Biogeographic Profile in Forensic Anthropology: An Alternative to Ancestry for Casework Along the United States-Mexico Border

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**Learning Overview:** After attending this presentation, attendees will be aware of how the place of origin can be inferred from unknown case variables and how place of origin in Mexico, trihybrid ancestry, case reporting date, and place of recovery on the border are interrelated. This study is relevant to the identification of Hispanics of Mexican origins, especially for Undocumented Border Crossers (UBC) casework from Arizona.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing biogeography as an alternative parameter to single-group ancestry. This presentation shows how novel geospatial and machine learning methods can be used to develop a biogeographic profile for unknown remains recovered along the United States-Mexico border.

Increasing fatalities and shifting demographics of Latin American migrants demand multi-focused research that works to improve rates of positive identification for UBCs.<sup>1-5</sup> Morphogenetic analyses of trihybrid ancestry have distinguished among many bio-cultural groups in Mexico, represented in UBC casework.<sup>6-11</sup> Demographic studies on migration networks have found distinctive transit pathways to “El Norte,” whereby migrants of different origins are associated with particular destinations along the United States-Mexico border at different times.<sup>12</sup> Predictions of individual sending region/origin are argued here to be more valuable for forensic case evaluation than single-group ancestry classifications.

While “ancestry” is an essential component of the biological profile, when defined as a single group category, such as Black, White, or Hispanic, its utility is limited in the border Identification (ID) context, as many UBCs will be classified as the same ancestry. Prior work has advocated for replacing single ancestry with probabilistic estimates of continental ancestry, which, under a trihybrid model, correspond to the relative quantities of European, Indigenous American, and African ancestry. It has shown that these ancestry proportions better account for admixture and the history of colonialism, slavery, and migration in the Americas.<sup>6-9</sup> Expanding this discussion to UBC casework, the present study rejects single ancestry classification in favor of reporting “biogeography”: an identity parameter that reflects the patterned association between ancestry proportions and geographic place of origin. It demonstrates how this “biogeographic profile” can be produced and mobilized in statistical models that predict location of origin from place of recovery. This information on sending region can direct the forensic case investigation by providing probable locations for finding next of kin and by reducing the pool of potential matches from missing persons lists.

Sampling ≈150 cases from the Pima County Office of the Medical Examiner (PCOME) in Arizona, finite-mixture, geospatial, machine learning, and traditional statistical methods are used to: (1) produce biogeographic profiles; (2) investigate the relationships among ancestry composition, geographic location of remains, and case-reporting date; and (3) build models that predict the individual’s documented origin as a microregional category or Geographic Information Systems (GIS) coordinates. Initial geospatial analysis shows global clustering relative to a random distribution (average nearest neighbor ratio=0.53), persisting at a range of scales (5–100km). Visual examination of mapped cases suggests clustering by both sending regions and ancestry, implying preset transit-corridors or routes. Autocorrelation analysis indicates clustering for trihybrid ancestries and latitudes/longitudes. Significant differences exist in ancestry quantities for paired microregions: Southeast cases carry more Indigenous and less European ancestry than North (+32, -27%) and Traditional (+25, -23%) cases. Correlations suggest that quantities of ancestry are structured by space/time. Individuals with more Indigenous ancestry originate in the southeast, more European ancestry in the northwest, and more African ancestry in the northeast. Individuals with higher African ancestry are more commonly recovered in earlier years. European and Indigenous ancestry are inversely related. Indigenous ancestry is a proxy for latitude, as it increases along a north-south axis and changes with the latitude-longitude of the recovery site. Tests of spatial relationships find that the north-south origin location is related to the north-south and east-west recovery location. Bootstrap forest methods confirm the significance of trihybrid ancestry and geo-temporal variables for predicting microregions and coordinates. While logistic regression explains only 38% of the variation and misclassifies 43% of cases, remarkable improvement is gained with more complex machine learning methods. The optimal neural network, fitted using k-fold validation, predicts place of origin with an overall model  $R^2=0.99$ . Latitudes and longitudes are estimated, respectively, with  $R^2=0.99$  and 0.93. Reducing the training sample, using 33% holdout-validation, produces a slightly lower overall model  $R^2=0.75$ .

This study provides a novel framework for producing a biogeographic profile and predicting individual place of origin using both biological and case-context information. It also identifies potential migration pathways that track from place of origin to place of recovery along the border and are related to trihybrid, especially Indigenous, ancestry. This study posits that patterns of spatio-temporal clustering (in case numbers, ancestry composition, reporting year, sex, origin) may reflect evolving motivations, collective knowledge, and barriers to migration. This information provides additional lines of evidence to direct case investigations or support partial identifications. Any new tool that holds the promise of increasing the number of UBC identifications is welcomed and will be put into practice at the PCOME.

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### Geospatial Mapping, United States-Mexico Border Dead, Predictive Models



## A175 A Geometric Morphometric Approach to Quantify the Impact of Admixture on Craniofacial Form

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**Learning Overview:** After attending this presentation, attendees will be familiarized with the implementation of two-dimensional geometric morphometric methods applied to a large sample of photographs in an attempt to understand how admixture may influence craniofacial form.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing a significant gap in available data for measuring the effect of admixture on ancestry analysis. Geometric morphometric methods for two-dimensional data permit an exploration of the differences in craniofacial morphology between various levels of admixed individuals.

Craniofacial form is under multidimensional selection borne as modular interactions, or functional morphotypic phenotypes. Multidimensional trait analysis can be used to quantify these interactions and, perhaps more importantly, may be used to construct statistical contrasts of the various forms—as *a priori* group designations—to one another in what is generally a high-dimensional shape space.<sup>1</sup> Geometric morphometric methods permit the exploration and quantification of this shape space as a single analytical unit.

This study uses a sample of digitized photographs from Caroline Bond Day's original thesis.<sup>2</sup> Day (1889–1948) was the first African American woman to obtain an advanced degree in anthropology, in any of the four fields.<sup>3</sup> As a Harvard/Radcliffe student, Day was mentored and influenced by E.A. Hooton, who published (and wrote the foreword to) Day's thesis, *A Study of Some Negro-White Families in the United States*.<sup>2</sup> Using a novel combination of sociological and anthropological data, Day examined aspects of biology and sociology within a sample of families having self-identified dual heritage, or admixture. More than 400 family photographs and individual portraits comprising data from 346 families—along with genealogical, physiological, and sociological data—are amassed within Day's thesis. These data are presented through the lens of admixture in the United States and the impact admixture had on sociological and biological aspects of each family. Day's dedication to data collection and her superb data management and reporting provide a dataset for a modern analysis of admixture and the influence of admixture on craniofacial form.

Many of the current research efforts devoted to studies of admixture focus on individuals of Hispanic ancestry and are thus of limited utility. Other works are based on such small sample sizes that the results are questionable, at best. To address these shortcomings, this study selected 197 photographs from Day's thesis with demonstrable head rotation angles between 0 and 10 degrees and that are free of objects (e.g., hair, shadows) obscuring facial landmarks.<sup>4</sup> To standardize pooling, Day's original assessments of “degree of blood quantum from Negro to White” were compressed: (1) 0–25%; (2) 26–50%; (3) 51–75%; and (4) 76–100%. Coordinate data for 24 soft tissue landmarks were collected and analyzed for each group using the geomorph package in R.<sup>5,6</sup>

A generalized Procrustes analysis was used to align and superimpose the landmark data and to calculate mean shape values for each group. These aligned data are used for all subsequent analyses. Multidimensional methods used to explore shape variability within and among various degrees of admixture include thin-plate splines, principal components analysis of the Procrustes landmark data to measure shape variability, and Procrustes Analyses of Variance (ANOVAs) with permutation to evaluate the observed patterns of shape variation and covariation within and among samples.

Several functional regions expressing shape differences were observed in the analysis. American Blacks had broader noses (nasal aperture width), narrower chins (gonial flare), and slightly wider zygomatics (bizygomatic breadth). However, identifying these differences required considerable magnification to visualize the shape differences (mag >x2). In fact, the Procrustes ordinary least squares ANOVA ( $p=0.436$ ;  $z=0.055$ ; permutations=1,000) indicate identifiable shape differences are not statistically significant. Additional explorations incorporating other levels of admixture were also insignificant.

The concept of admixture in the United States reflects historical events, such as forced relocation of Africans and Native Americans to and within the United States.<sup>7</sup> The continuing levels of admixture can reveal fine-scale genetic differences in ancestry, but these differences may not be expressed in skeletal tissues. In fact, the very concept of “admixture” represented in morphological characters is typological and as such is ill-advised as a descriptor for analyses. Larger samples of known individuals of self-reported levels of genetic admixture are needed before any assertions regarding admixture estimation in skeletal remains can be made.

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### Forensic Anthropology, Ancestry, Admixture

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### A176 Craniometrics vs. Single Nucleotide Polymorphism (SNP)s: Is There a Correlation?

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**Learning Overview:** After attending this presentation, attendees will understand the relationship between craniometrics and specific DNA variations (SNPs) and its value for positive identification purposes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by investigating specific regions of the human genome to determine how they influence the craniofacial skeletal structure in an effort to improve human identification and facial reconstruction efforts for positive identification of the deceased.

In a world where forensic countermeasures are sophisticated tools to deflect, detect, or solve a crime, it is important to consider the human aspect in forensic science. Human identification has long been a leading tool to hold perpetrators accountable, give families closure, and put a face on a skull. This project is a pilot study to merge three disciplines that fall under the human identification umbrella: forensic anthropology, forensic genetics, and forensic art. Current facial research in genetics focuses on data from living individuals where specific SNPs that influence specific regions of the face have been found.<sup>1-3</sup> This study determines if these same regions can be translated to craniometric dimensions (inter-landmark distances) of the underlying skull itself.

The goal of this project is to provide information regarding the correlation of craniometric measurements and SNPs, as well as how these relationships can decrease subjectivity in forensic facial reconstructions and encourage interdisciplinary work within the forensic sciences. This study examined a selection of candidate SNPs currently identified in the literature to determine if there were correlations between inter-landmark distances and those SNPs within the same individual. Craniometric landmark data were collected from 17 documented skulls from the Texas State Donated Skeletal Collection using a 3D MicroScribe® digitizer. Criteria for inclusion in this study included European ancestry, the presence of intact skulls, and the presence of associated donor blood cards collected at the time of body donation. Using these blood samples, DNA from each individual was extracted, amplified, and sequenced through next generation sequencing for the specific chosen SNPs. Afterward, bioinformatics tests were applied to observe the presence or absence of the major or minor alleles in the specific locations on the genome. After determining the presence or absence of an SNP, a set of statistical tests were performed, including: Spearman's correlation between the craniometric measurements and the individual's genetic data variables; two-way hierarchical clustering and Bootstrap Forest modeling between variables that demonstrated significant correlation; a principal component analysis was performed on the craniometric data (inter-landmark measurements) and genetic data (SNP presence/absence) in order to check the homogeneity of each data set; and finally, a pair-wise Procrustes analysis was completed on the correlation of the two data sets as different groups.

The results indicate a correlation in various degrees between the targeted craniofacial regions and the targeted SNPs. There were 11 SNPs that showed significant correlation ( $p < 0.05$ ). However, the correlations were not as expected and showed some interesting results. By group level, there is no significant correlation; however, there is correlation at the individual level. While some SNPs affected the soft tissues only, others showed correlations with the skull (hard tissue), a finding that had not been previously known. These results show that combining craniometric and DNA analyses may provide new tools for forensic anthropological, genetic, and facial artistic approaches to human identification.

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#### Forensic Genetics, Forensic Anthropology, Human Identification



## A177 A Call for a Paradigm Shift in the Study of Ancestry

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**Learning Overview:** After attending this presentation, attendees will understand the underlying factors responsible for human variation with implications for ancestry estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a new paradigm for a better understanding of ancestry.

The estimation of ancestry is a crucial component in human identification but one of the most difficult parameters of the biological profile. Recently, antiquated and over-simplistic views of ancestry based on the trifecta of continental populations from Asia, Europe, and Africa have regained popularity. However, this facile presumption ignores underlying microevolutionary forces such as gene flow, drift, and migrations that are responsible for human diversification. Global population studies demonstrate that human craniofacial morphology fits a neutral evolutionary model because contiguous populations more frequently exchange genes and/or share common ancestry.

There is a need for broad synthesis to better understand the underlying patterns of modern human variation that will inform the estimation of ancestry. The purpose of this study is to explore craniofacial variation in Latin America and test the validity of using the tri-continental approach to ancestry. The sample totals 397 individuals, and samples were separated by biological sex when both were available for analysis (Chile: females  $n=34$ , males  $n=37$ ; Colombia: females  $n=11$ , males  $n=53$ ; Cuba: males  $n=19$ ; Enslaved Africans from Cuba: males  $n=25$ ; Guatemala: males  $n=71$ ; Puerto Rico: males  $n=5$ ; Panama: males  $n=10$ ; Peru: males  $n=7$ ; Spanish: females  $n=58$ , males  $n=67$ ). Sixteen standard Type 1 and Type 2 craniofacial homologous coordinate landmarks were used in the analysis. Coordinate data must first undergo a Generalized Procrustes Analysis (GPA) transformation before subsequent statistical analyses can be performed. The shareware program MorphoJ was used to translate, rotate, and scale all coordinates into a common coordinate system using GPA. Shape is defined as all of the geometric information that remains after the effects of location, scale, and rotational effects are removed. Centroid size is a measure of geometric scale that is mathematically independent of shape. A Principal Component Analysis (PCA) of the covariance matrix was conducted on the GPA-transformed coordinates to reduce dimensionality of the data for subsequent multivariate statistical analyses. Shape and size variation among the groups was examined using a Procrustes Analysis of Variance (ANOVA). Among-group variation was examined using Canonical Variates Analysis (CVA). Mahalanobis distance or generalized distance, which considers the correlations among variables when computing the distance between means, was used to examine group relatedness. An average linkage hierarchical (or agglomerative) cluster analysis was performed using the generalized distance matrix to examine group similarity. Hierarchical clustering begins with every sample in a single cluster, then in each successive iteration, it merges the closest pair of clusters (distances between all pairs and averages all these distances) until all the data are in one cluster. The cluster analysis was performed in JMP® Pro 14.

The Procrustes ANOVA results show significant group variation for shape ( $F(242, 15,785)=6.82, p<.0001$ ) and centroid size ( $F(11,385)=22.35, p<.0001$ ). Ninety-one percent of the total variation is accounted for on the first five canonical variates. The generalized distance results show that all groups are significantly different from one another based on 1,000 permutations ( $p$ -values range from  $0.01<p<.0001$ ), except for Puerto Rico and Peru ( $D=4.14, p$ -value= $0.347$ ). The dendrogram produced from the hierarchical cluster analysis shows two distinct clusters: Chile/Spain. Panama, Cuba, Guatemala, and Colombia branch off the Chile/Spain cluster. The enslaved African sample clusters with Peru, and Puerto Rico is the most dissimilar. These results demonstrate that craniofacial morphology is much more complex than mere proportions of Asian, African, and European continental groups. Notably, these results are consistent with isolation-by-distance of early populations in the region and historical migrations, which underscores the need for a paradigm shift from a three-way continental approach to a population history approach to ancestry.

### Ancestry, Paradigm Shift, Geometric Morphometrics



## A178 Ancestry Estimation, Race Science, and Scientific Racism in Forensic Anthropology

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**Learning Overview:** After attending this presentation, attendees will understand how forensic anthropological research influences the production of race science and is used in the perpetuation of racist ideologies. Additionally, results from an online survey are presented regarding the views of biological anthropologists on race and ancestry, and professional obligations in response to race science and scientific racism.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the implications of forensic anthropological research and practice in race science and the enforcement of racial hierarchies. Such knowledge, in conjunction with current views in biological anthropology concerning race and ancestry as well as ethical practice, may be used to establish a series of best practices regarding language use, research design, and public engagement.

In forensic anthropology, practitioners differentiate the concepts of biological ancestry and social race to argue that typological views are no longer a valid perspective in the discipline. However, despite these efforts, individuals in a variety of fields continue to assert the existence of biological racial differences with a resulting natural hierarchy. Importantly, for the anthropological community, the research is misused to promote these racist views. To understand this misappropriation, this project examines the literature for instances in which proponents of race science and White supremacists/nationalists utilize forensic anthropological research to justify the existence of biological races. Articles in multiple journals were reviewed, including *Mankind Quarterly*; *The Journal of Social, Political, and Economic Studies*; *American Renaissance*; and *The Occidental Quarterly*.

Two primary ways of misusing research on skeletal human variation were identified. The first is the use of forensic ancestry estimation as evidence for the existence of biological races with clearly delineated differences. The second is the use of phenotypic traits as correlates for racial differences in relation to personality, behavior, and intelligence. Indirectly, research in age and sex estimation, including studies of dental and skeletal development, were used to support various claims of racial differences in intelligence and temperament.

To supplement this review of the literature on race science, results from a recent online survey approved by the Institutional Review Board of the University of Nevada, Reno ( $n=257$ ) are discussed. In this survey of biological anthropologists, there is a consensus regarding the social versus biological dichotomy of race and ancestry. However, there is disagreement as to whether ancestry itself is also a social construct (28.05% agree vs. 49.32% disagree). There is a lack of agreement as to whether the use of categories like African American and European involves the identification of a biological race (21.83% agree) and if forensic anthropology reinforces racial typology (27.95% agree). In fact, scholars are almost evenly split as to whether using categories like African American or White are justified when studying phenotypic variation, with 32.54% agreeing and 35.32% disagreeing.

Questions regarding terminology show some disagreement between respondents. Biological anthropologists suggest geographically based terminology (e.g., European) is preferred over socially based terms (e.g., White) (54.63% vs. 9.73%). There is slightly more disagreement regarding social/nationality-based terms like European American (24.55% agree vs. 42.41% disagree). There is also some debate regarding the use of Hispanic/Latino as an ancestry category with approximately 26% arguing it is a valid category and about 43% arguing it is not.

Nearly half of respondents argued that political correctness is a problem in biological anthropology (46.12%) and about one-quarter of respondents agreed that concerns over the moral and ethical implications of research (e.g., photos, ancestry, terminology) result in the silencing of research. Most anthropologists argue that if misappropriation of research occurs, there is an ethical obligation to intervene (94.87%). Finally, despite these concerns, 71.6% of respondents argued ancestry estimation should remain a component of forensic practice.

The review presented here provides important evidence of continued weaponization of forensic anthropological research to push extremist and harmful ideologies of racial typology. Based on this study, it is critical that forensic anthropologists clearly evaluate what is meant by ancestry and its relation to race. Further, they should be mindful of the terminology used, reference sample construction, and method design. Research should be transparent and forensic anthropologists should engage with the public in discussions of race and biology as well as work to combat the misappropriation of their research.

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### Ancestry Estimation, Race Science, Scientific Racism

## B1 Complex DNA Mixture Analysis: Massively Parallel Sequencing (MPS) of Rare Single Nucleotide Polymorphisms (SNPs)

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**Learning Overview:** After attending this presentation, attendees will understand how using MPS of rare SNPs allows the matching of more than three references to complex mixtures of DNA, including those obtained from touch DNA.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing experimental evidence from two independent laboratories that demonstrates the power of using MPS of rare SNPs for the analysis of complex mixtures.

DNA mixtures from three or more contributors have proven difficult to analyze using the current state-of-the-art method of Short Tandem Repeat (STR) amplification followed by Capillary Electrophoresis (CE). This is due to multiple issues, including the sharing of alleles between different contributors, the production of confounding stutter products during amplification, and the small amount of DNA recovered from some contributors, causing allele drop-out. The discriminatory power of STRs lies in the fact that they are highly polymorphic, even though the individual alleles are not necessarily rare. This feature has enabled forensic scientists to streamline their STR analysis workflow to tens of loci in the genome. In contrast, there are only two alleles at each SNP locus used in this study, and the presence of each minor allele in an individual is relatively uncommon. Therefore, more SNP loci are required to produce a unique individual signature, and many more SNP loci are required for successful mixture analysis.<sup>1</sup> Although the SNP loci used in this study are also affected by allele sharing and drop-out, the drop-in rates for single nucleotides are relatively low, there are no stutter artifacts, and many more loci can be analyzed simultaneously, producing statistical power that enables detection of individual contributors in complex mixtures.

SNP loci were selected based on low fixation index ( $F_{ST}$ ) values and low global minor allele frequencies. In addition, certain SNPs were chosen from the literature for other purposes (biogeographic ancestry, surname and phenotype prediction, identification).<sup>2-7</sup> This panel of 18,147 loci was submitted to Ion Torrent™ AmpliSeq™ White Glove Design service, and primers were successfully designed for 14,934 loci (14,731 amplicons) in a single-tube amplification format. The resulting panel of primers was tested by sequencing DNA from 178 individuals, and 13,917 of these primers (approximately 93%) produced amplicons with adequate sequence coverage at default quality scores. Within the large primer panel containing 13,917 primer pairs, only a subset of loci is appropriate for analysis of DNA mixtures. The panel to be used for mixture analysis was selected from the larger primer panel based on specific criteria ( $F_{ST} \leq 0.08$ , minor allele frequency  $\leq 0.3$ , number of reads within a ten-fold range, strand bias ratio  $> 0.5$ , minor allele ratio for homozygous major loci  $\leq 0.005$ , no Mendelian errors, distance of  $\geq 500,000$  base pairs between loci). Primers meeting these criteria created a mixture panel targeting 2,655 loci, of which 2,311 are used for mixture analysis, while an additional 344 loci were included for identification and biogeographic ancestry prediction.

A custom software platform, IdPrism, was developed with a simple user interface and is composed of modules that identify sequence variants (minor alleles), as well as perform identification, mixture analysis, determine familial relationships, phenotypes, and Bio-Geographic Ancestry (BGA).<sup>8-13</sup>

Two independent laboratories conducted experiments with both controlled, laboratory generated mixtures, and realistic touch samples. A dilution series of single-source samples demonstrated the sensitivity of MPS, where reference profiles can be identified 100% of the time with 20pg of input and 50% of the time with 5pg input. In addition, the minor contributor can be identified at a minor to major ratio of 1:100 for 1<sup>st</sup> degree relatives. Several mixtures were prepared from saliva specimens to simulate complex forensic mixtures (e.g., two- to ten-person mixtures) in various proportions and total template amounts to identify potential limitations of the method. Results from these mixtures demonstrate the ability to resolve up to ten-person mixtures with as low as 1ng total input DNA as well as 1<sup>st</sup> degree relatives. In addition, results from touch samples demonstrated that as many as ten people can be identified in a single mixture, depending of the detection threshold that is applied. Even at a stringent threshold of Probability of Random Man Not Excluded P(RMNE) of  $10 \times 10^{-9}$ , 35 touch samples with four or more individuals were correctly identified as contributors.

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### DNA Mixtures, Touch DNA, DNA Sequencing

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### B2 Implementing Hematoxylin Into Casework at the North Carolina State Crime Laboratory

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**Learning Overview:** After attending this presentation, attendees will understand the growth phases of human hair, the type of hair that may be left behind at a crime scene, and how the presence of telogen hair roots can potentially impact DNA results obtained from hair.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of the internal hematoxylin study performed at the North Carolina State Crime Laboratory and how implementation of the hematoxylin staining technique will ensure that only hair roots with the best potential to develop a DNA profile are sent for DNA analysis.

When a hair root is sent for DNA analysis, the hair examiner has determined that this hair may provide valuable information to the investigators. Hair analysis and DNA analysis complement each other in that DNA may be able to provide a potential identification for the source of a hair that the examiner deemed important. However, sending a hair root for DNA analysis is a destructive test and no further information can be obtained from that root if a profile is not developed. The hair examiners in the trace evidence section noticed over the past several years that hair roots being sent for DNA analysis were not yielding DNA profiles as expected. The recent advancements in the forensic biology section's detection limits prompted the trace evidence hair examiners to begin researching whether changes needed to be made to the current hair root removal protocol to increase the likelihood of developing a DNA profile from a hair root.

Several staining methods for determining the presence of nuclei within a hair root have been tested and published in scientific journals, as well as validated within other crime laboratories. Based on these studies, the trace evidence hair examiners decided to validate the method of hematoxylin staining for use in screening roots in the telogen growth phase for DNA analysis. In this study, more than 700 head hairs from approximately 15 living donors were examined for the presence of telogen roots. Those roots were then stained using hematoxylin and examined for the presence of nuclei. The roots were separated into one of six groups based on the number of nuclei present: Group 0 (0 nuclei), Group 1 (1 to 10 nuclei), Group 2 (11 to 20 nuclei), Group 3 (21 to 30 nuclei), Group 4 (31 to 40 nuclei), and Group 5 (41 or greater nuclei). A set of 64 hair roots, including the negative control group (Group 0) and a positive control group (anagen or catagen growth phase hair roots), were sent for quantitative analysis in the forensic biology section. The quantitative data showed a clear delineation between Groups 1 and 2, where 36% of Group 1 verses 80% of Group 2 passed the quantification cutoff. All samples in Group 0, Group 1, and the positive control group were then amplified along with a representative sample in Groups 2, 3, 4, and 5. After amplification, the delineation between the results of Groups 1 and 2 maintained constant with 27% of Group 1 obtaining DNA profiles verses 89% of Group 2 obtaining DNA profiles. This showed that the cutoff for the minimum number of nuclei required in order to obtain a potential DNA profile at the North Carolina State Crime Laboratory is 11 or more nuclei.

#### **Hair, Hematoxylin, Telogen**

### B3 The “Secondary Burial” in Southern Italy: How Traditional Funerary Practices Affect DNA Preservation in Forensic Human Identification

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**Learning Overview:** After attending this presentation, attendees will be able to describe how burial practices affect DNA preservation for forensic purposes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the handling of human remains in South Italian cemeteries may promote the preservation of human bones and, hence, improve the analysis of degraded DNA.

Death represents the definitive transition from life to afterlife, from one social status to another. These transitions are part of different cultures, and it is possible to notice them every time a member belonging to a society dies. Burial rituals are among the few tangible forms of mortuary customs that reflect the belief in the afterlife. These procedures are the result of a complex mix of factors: cultural, philosophical, and practical (e.g., body handling, grave location, and cemetery organization).<sup>1</sup>

In Southern Italian tradition, the deceased person is perceived to be in a transition stage, between life and death, until the burial ceremony takes place. To verify that the transition to the afterlife was successful, from the 18<sup>th</sup> century on, a so-called “secondary burial” has been carried out. During the first stage (as soon as the person dies), cemetery attendants bury the corpse in a sealed wooden coffin. Then, after five to ten years, the morticians exhume the corpse for the definitive allocation. At this stage, the deceased’s relatives take care of the remains. They wash, disinfect, and wrap the human remains in a linen sheet and finally bury the deceased in a stone niche. The whole procedure is performed in compliance with Italian Presidential Decree no. 285, September 19, 1990.

If parentage or any other forensic tests are required, the Judicial Authority allows experts (e.g., forensic geneticists, pathologists, anthropologists) to perform the sampling and analysis. According to the time since death, the remains are recovered from the burial site in different stages of decomposition.

For the present study, four decomposition stages have been recognized: Fresh, Bloated, Advance Decay, and Skeletonized/Mummified.

Although DNA extraction can be conducted in any area of the body, the femoral compact bone represents the best suitable tissue, especially when DNA is degraded due to postmortem phenomena.<sup>2,3</sup>

A review of 150 analyses performed from 2001 to 2018 is presented here to evaluate how the different decomposition stages can affect both the quality and concentration of the DNA extracted from human remains that underwent a “secondary burial.”

As expected, the quality and quantity of extracted DNA decreased with the decomposition process; however, all the Short Tandem Repeat (STR) genetic profiles were adequate for forensic purposes.<sup>4-7</sup> These results demonstrate how the traditional “secondary burial” allows for better preservation of the genetic evidence compared with more modern mortuary customs.

The goal of this study is to evaluate how the handling of human remains in Southern Italian cemeteries positively affects the preservation of human bones and, hence, improves DNA analysis. These results are particularly relevant for the experts involved in exhumations related to parentage tests and identifications of an individual for both recent and cold cases.

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#### Forensic Science, DNA Analysis, STRs Identification



## B4 The Student Experience in Participating in a Collaborative Exercise for Methylation-Based Body Fluid Identification

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**Learning Overview:** After attending this presentation, attendees will better understand forensic epigenetics and the potential of using DNA methylation for the identification of biological fluids. Attendees will also gain a better understanding of the student experience in implementing an established Multiplex SNaPshot™ assay and interpreting body fluid identification results via methylation profiles for the purposes of an interlaboratory collaborative exercise.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing results from a student evaluation of a novel methylation-based Multiplex SNaPshot™ assay for body fluid identification. The assay has strong potential to be implemented in a general forensic laboratory as a confirmatory test. This would prevent the need to perform time-consuming serological testing to identify semen and offer a confirmatory assay for the identification of saliva for which there is currently not a test commonly used by crime labs in the United States. This assay also has the potential to be automated using equipment well-established in forensic laboratories and to be used for the identification of biological fluid mixtures.

Epigenetics is defined as reversible heritable changes in gene expression that do not alter the DNA sequence itself. Epigenetic changes play an important role in gene expression and can be altered due to a person's age, lifestyle, and environment.<sup>1</sup> DNA methylation, a DNA base modification, is the process by which methyl groups are added to cytosines on the DNA. These sites are termed CpG sites. DNA methylation patterns are established through cell division, giving various cells and tissue types a specific DNA methylation profile, and these patterns have been thoroughly studied and are regarded as a promising tool for forensic science research, including age prediction and body fluid identification.<sup>2</sup> DNA methylation can be quantified to produce methylation profiles via a process known as bisulfite conversion. In this process, un-methylated cytosine residues are converted to uracil, and during Polymerase Chain Reaction (PCR) are then converted to thymine. Following PCR amplification and single-base extension, the resulting amounts of cytosine and thymine correspond to the amount of methylation present at a specific CpG site. Bisulfite conversion is considered the gold standard in assessing DNA methylation, proving to be successful using small amounts of DNA (50–100pg) and result in 80% DNA recovery.<sup>3</sup> The accurate identification of various body fluids using a methylation-based assay will aid forensic investigators in linking evidence and acts of crime where current assays may yield a false-positive or false-negative result.

In this collaborative study, samples from Seoul National University College of Medicine were sent to various labs, including The George Washington University Department of Forensic Science, to be analyzed using an established Multiplex SNaPshot™ assay for body fluid identification.<sup>3</sup> Samples include swabs of unknown tissue source, genomic DNA of known and unknown tissue source, bisulfite-converted DNA of known tissue source, and reference Single Base Extension (SBE) products. Methylation profiles were comprised of a panel of nine CpG sites (cg17610929, cg26763284-138d, cg06379435, cg08792630, cg09765089-231d, cg26079753-7d, cg09652652-2d, cg18069290, and cg09696411). These CpG sites were chosen based on their specificity to blood, semen, and saliva.<sup>2</sup> The panel includes two CpG sites for semen, two for blood, one for saliva, two for vaginal fluid, and two for menstrual blood. Appropriate samples were extracted on the QIAGEN® QIAamp® DNA Investigator Kit, quantified on the Quantifiler® Trio, then processed via bisulfite conversion on the QIAGEN® EpiTect™ Fast DNA Bisulfite Kit, multiplex PCR, and multiplex SNaPshot™. SBE products were then analyzed on the Applied Biosystems® 3500 Genetic Analyzer to produce methylation profiles. Each profile shows peaks for each of the nine CpG sites, indicating methylation status indicative of either semen, saliva, or blood.

All samples produced reliable results using 40ng of input DNA for the bisulfite conversion process. Methylation profiles produced results consistent with blood, semen, and saliva from the corresponding samples. In addition, three genomic DNA samples and three swabs of unknown tissue source produced a methylation profile consistent with blood, semen, and saliva. Observed peak heights for CpG sites cg06379435 and cg08792630, which correspond to blood, and site cg09652652-2d, which corresponds to saliva, were present but lower than expected. Preliminary results indicate the multiplex SNaPshot™ assay for body fluid identification can be successfully performed by analysts familiar with the protocol and carried out using instrumentation that is well-established in general forensic laboratories.

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2. Lee H.Y., Park M.J., Choi A., An J.H., Yang W.I., Shin K.J. Potential forensic application of DNA methylation profiling to body fluid identification. *International Journal of Legal Medicine*. 126 (2012) 55-62.
3. Vidaki A., Keyser M. Recent progress, methods and perspectives in forensic epigenetics. *Forensic Science International: Genetics*. 37 (2018) 180-195.

### Biological Fluid Identification, DNA Methylation, Bisulfite Conversion

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**B5 A Student Experience Evaluating the Ability of a Methylation-Based Age Prediction Model to Be Implemented in the Forensic Laboratory as Part of a Collaborative Study**

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**Learning Overview:** After attending this presentation, attendees will better understand the students' experience in practicing a multiplex SNaPshot™ assay and a Methylation-Sensitive Restriction Enzyme (MSRE) Polymerase Chain Reaction (PCR) assay for methylation-based age prediction. Attendees will understand the advantages and the disadvantages of these two methods in forensic applications for methylation analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the results from practicing and evaluating a multiplex SNaPshot™ assay as well as a preliminary test of an MSRE PCR method for methylation-based age prediction. Both procedures have a strong potential to be implemented in a general forensic laboratory. These DNA methylation-based age prediction methods would provide useful information to narrow the search for suspects when the profile from evidence samples does not match any of the reference samples or any profile in the available database.

DNA methylation analysis is reported to be a promising tool for age prediction for forensic applications. DNA methylation is a form of epigenetic regulation of gene expression that widely occurs on the CpG sites of the genome. As age-associated DNA methylation is tissue specific, most age prediction models developed are specific to the type of body fluid tested. This study focuses on the prediction for blood samples, the most commonly found body fluid at the crime scene. The commonly used methods to detect differential DNA methylation at a CpG site of interest are bisulfite-based approaches and MSRE PCR analysis. Bisulfite conversion-based approaches, including pyrosequencing, SNaPshot™-based Single Base Extension (SBE), and massively parallel sequencing, are the most commonly used in forensic research to date. Age prediction models have been proposed using methylation status obtained from bisulfite-conversion approaches, including a multiplex SNaPshot™ assay for blood samples based on the methylation status of five CpG sites from ELOVL2, FHL2, KLF4, C1orf132, and TRIM59 genes developed by Lee et al.<sup>1</sup> The alternative method for methylation analysis is MSRE PCR analysis. This approach utilizes the differential cleavage ability of MSRE depending on the methylation status of the CpG site of interest. The MSRE approach depends on specific recognition sites that flank the CpG site. Compared to bisulfite conversion, MSRE requires less DNA input, which could be a huge advantage for forensic investigations with a limited amount of sample.

This study aims to evaluate bisulfite-based SNaPshot™ assay and MSRE PCR analysis in successfully estimating the age of an individual. For bisulfite-based approach, the study practiced with the age prediction model developed by Lee et al. as a part of a collaborative study to demonstrate its accuracy in age prediction and ability to be implemented in general forensic laboratories.<sup>1</sup> This method was tested on familial samples of the children versus their corresponding parents. Preliminary results show that this model correctly grouped the samples into the corresponding age group. A sample of a 2.5-year-old was also tested, and the age prediction was 2.28. For MSRE PCR analysis, five age-associated CpG sites that can be recognized and cleaved by the methylation sensitive restriction enzyme HhaI were selected to develop an age prediction model. These sites are reported to have strong correlation between their methylation status and age in blood samples. This study demonstrates an effective procedure for MSRE-based analysis consisting of DNA extraction, quantification, MSRE digestion, PCR amplification, and capillary electrophoresis. The preliminary results show that the methylation status at locus cg23500537 is significantly different between child and adult samples.

**Reference(s):**

- <sup>1</sup>. Jung S.E., Lim S.M., Hong S.R., Lee E.H., Shin K.J., Lee H.Y. DNA methylation of the ELOVL2, FHL2, KLF14, C1orf132/MIR29B2C, and TRIM59 genes for age prediction from blood, saliva, and buccal swab samples. *Forensic Science International: Genetics* 28 (2019) 1-8.

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**DNA Methylation, Age Prediction, Bisulfite Conversion and MSRE**



## B6 A Comparison of InnoQuant® and Quantifiler® Trio as Quantification/Degradation Methods for Predicting Next Generation Sequencing (NGS) Success

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**Learning Overview:** After attending this presentation, attendees will better understand the relationship between DNA quantification/degradation for forensic-type samples and NGS library yield as it relates to NGS success in obtaining a full profile of Short Tandem Repeat (STR) loci.

**Impact on the Forensic Science Community:** The results in this presentation will impact the forensic science community by providing information from DNA quantification/degradation that will streamline analysis by helping the analyst to decide to pursue STR or mitochondrial DNA (mtDNA) analysis.

DNA quantification kits can not only provide information on the amount of DNA in a sample, but also on the state of its degradation. Studies have been reported on the accuracy and reproducibility of the InnoQuant® and Quantifiler® Trio quantification methods in comparison with other commercially available DNA quantification kits capable of degradation estimation.<sup>1,2</sup> The Degradation Index (DI) is a ratio of a short and long fluorescent fragment with degraded DNA giving ratios >1. However, there has not been a report on an evaluation of the DI in conjunction with NGS library preparation success rates. This research on DNA quantification will give biologists a better assessment for how challenging samples will perform when subjected to library preparation, the first step for NGS processing. In addition, knowing the DI helps the analyst predict the success of obtaining a full STR profile by conventional Capillary Electrophoresis (CE), but it is not known if this is true for NGS as well.<sup>3</sup> Analysts can use this information to decide whether to pursue mitochondrial DNA (mtDNA) sequencing, which would be more successful in providing information on the source of the sample than STR analysis.

In this work, DNA from samples of blood (5µL homogenized whole blood; treated with either sonication or Ultraviolet (UV) irradiation in an attempt to artificially degrade the DNA; n, 24), bone (50mg homogenized powder; n, 12), and hairs (1cm fragments cut from root end; n, 12) from three donors each was extracted following standard procedures and the DNA was subjected to quantification and degradation analysis in duplicate using the InnoQuant® (IQ) and Quantifiler® Trio (QT) quantification kits. Library preparation, amplification, and indexing was performed using the MiSeq™ FGx Forensic Genomics System with Ing DNA and Primer Mix B multiplex. After purification, libraries were quantified using the PowerSeq™ Quant MS System. From 90 libraries, 30 were selected to be normalized, pooled, and sequenced on the MiSeq™ FGx instrument with the MiSeq™ ForenSeq™ Sequencing Kit. Sequencing data was analyzed using the ForenSeq™ Universal Analysis Software (UAS) to produce autosomal STR profiles using the manufacturer's default settings (analytical threshold, 4.5% and interpretation threshold, 7.6%).

No correlation was observed between the library yield and the level of the DI in the ranges obtained for both kits. Blood DI values ranged from 0.72–1.8 (IQ) and 0.80–1.7 (QT); bone DI ranged from 2.5–15.9 (IQ) and 1.8–6.8 (QT); and hair DI ranged from 0.76–4.74 (IQ) and 0.76–4.57 (QT). Linear regression analysis was performed to determine whether a relationship existed between the reported DI for both kits and NGS library yields. Results showed weak linear relationships for all sample types from both kits ( $R^2$ : 0.0487 [IQ] and 0.1175 [QT] for blood; 0.2167 [IQ] and 0.0926 [QT] for bone; and 0.2176 [IQ] and 0.0495 [QT] for hair).

The amount of DNA used to prepare the library strongly influenced both the library yields and NGS success. Average library yields ranged from 145,000pM (1.0ng), 4,700pM (67–90pg), 3,000pM (1–28pg) and 2,400pM (<1pg). The sequencing success was judged by the percentage of STR loci (n, 28) reported by the NGS analysis software. For example, 99–100% of the STR loci were reported when 1.0ng was used to prepare the libraries from blood and 82% from bone. However, with decreased input, fewer loci were reported (80pg bone: 35–96%; 30pg hair: 71–93%; 8pg hair: 4%).

In summary, this study demonstrated that NGS STR genotyping success appears to be influenced more by the amount of DNA used to prepare the library than by the degradation state of the DNA. The two quantification kits behaved similarly with respect to the DI.

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3. LeFebvre A.K., Ayers S., Gahn L., Sinha H., Brown H., Murphy G., et al. Improving STR profile success rates for property crime specimens using InnoQuant Human DNA Quantitation & Degradation Assessment Kit. *J. Forensic Investigation* 2017;5:1-9.

### NGS-STRs, DNA Quantitation, DNA Degradation



### B7 Further Evaluation of a Dry Vacuuming Technique for Recovery of DNA From Handwritten Documents

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**Learning Overview:** After attending this presentation, attendees will know more about a non-destructive method of vacuum swabbing large surface areas of handwritten notes and will have learned how it affects the workflow for paper evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing validation data on a method that allows the investigator to process paper evidence for DNA prior to latent print and questioned document examination. This will increase success rates and reduce contamination events for DNA recovered from handwritten documents.

In a previous presentation, a non-destructive homemade vacuum method for collecting biological material from handwritten documents was introduced.<sup>1</sup> The method had yielded >70% of useful DNA profiles and did not negatively affect friction ridge detail and indented writing. Based on these results, it should be possible to reverse the current forensic laboratory practice of first processing paper for indented writing, then for latent prints, and only collect DNA as the last step from areas with visible prints. Aside from the risk of contamination and loss of DNA, this workflow has the disadvantage of not including any DNA evidence deposited during the writing process and the writer's hand scraping across the page. The previous work had been performed on standard white copy paper.<sup>1</sup> Prior to casework implementation, the method needed to be tested under different conditions (e.g., on different paper substrates). Accordingly, this small validation study was designed to cover other paper types, such as notebook paper, bank deposit slips, magazine pages, and manila envelopes.

Paper substrates were made DNA free by Ultraviolet (UV) exposure in an Air Science UV-Box™. Male volunteers consenting to be part of the study were asked to write a standard text and deposit left-hand fingerprints on each piece of paper. The same set of eight volunteers was used for all paper types. The writing samples were suspended using adjustable magnetic clamps and then vacuumed using a Carolina® 9-inch glass pipette containing a moistened cotton Puritan® swab with a vacuum hose attached to the narrow end of the pipette. DNA was extracted using a lysis buffer with 5% Chelex®, 10% Tween® 20, and Proteinase K followed by concentrating the extract with DNA Fastflow Millipore® Microcon membrane filter units.<sup>2</sup> All recovered samples were quantified using Quantifiler® Trio and typed with AmpFℓSTR® Identifiler® Plus, both from Thermo Fisher Scientific™ Applied Biosystems®. Fingerprints were developed with 1,2 Indanedione in a petroleum ether zinc chloride solution.

The quantity of recovered DNA varied from donor to donor, but showed a trend that can be explained by the different sizes and surface properties of the tested paper types. Copy and notebook paper had the same size, but copy paper is rougher and yielded more DNA. Deposit slips with their smooth surface and small size had a very low DNA yield. The vacuum did not work well on manila envelopes as these were too thick to allow for air to pass through and the vacuum had less suction. Magazine paper is very thin and was difficult to handle. Fingerprints were successfully detected on copy paper, deposit slips and manila envelopes, but not on magazine paper. Friction ridge detail quality was not affected by the vacuum collection process.

#### Reference(s):

1. McLaughlin P., Prinz M. Improved DNA recovery from handwritten documents. *Proceedings of the American Academy of Forensic Sciences*, 71<sup>st</sup> Annual Scientific Meeting, Baltimore, MD. 2019. Abstract B114, p318.
2. Forsberg C., Jansson L., Ansell R., Hedman J. High-throughput DNA extraction of forensic adhesive tapes. *Forensic Sci Int Genet.* 2016; 24:158–63.

#### DNA Recovery, Paper, Fingerprints



### B8 Eye and Skin Color Identity Single Nucleotide Polymorphisms (SNPs) Screening Using Polymerase Chain Reaction (PCR) High Resolution Melt (HRM) Assays

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**Learning Overview:** After attending this presentation, attendees will understand the advantages of using a single-step assay to screen eye and skin color phenotypic characteristics for unknown samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing screening tools that can be used post-PCR, and potentially within a quantitation assay, to determine phenotypic traits of eye and skin color, potentially eliminating the need for additional post-PCR assays. The screening assays can also be used to check sequencing results that are difficult to interpret.

Determining phenotypic characteristics can be extremely beneficial in forensic investigations, providing information to aid in identifying unknown suspects or missing persons or in corroborating eyewitness testimony.<sup>1,2</sup> This tool, commonly called Forensic DNA Phenotyping (FDP), can also aid in forensic facial reconstruction by providing phenotypic traits or pigment-related features of unidentified skeletal remains that would previously not be known.<sup>1-3</sup> SNPs are the key to FDP and are used to predict eye and skin color using SNPs that have been previously linked to phenotypic gene expression.

One forward and two reverse PCR primers were designed for each of six SNPs. Each SNP has a major and minor allele, and was differentiated by the two reverse primers, one with a GC-clamp to shift the melt temperature higher and one without. Proper PCR amplification was checked using several DNA standards including 2800M, K562, 9948 that were previously sequenced using the Verogen ForenSeq™ DNA Signature Prep Kit using Primer Set B on the MiSeq® FGx through Massively Parallel Sequencing (MPS). The targeted SNPs included rs12913832 (HERC2 gene), rs12203592 (IRF4 gene), rs12896399 (SLC24A4 gene), rs16891982 (SLC45A2 gene), rs1426654 (SLC24A5 gene), and rs885479 (MC1R gene). Following PCR, an HRM assay was performed using a Rotor-Gene® Q real-time PCR instrument. The QIAGEN® ScreenClust® software was used to identify allele(s) present based on their differing melting temperatures and clustering. After the primers were optimized for accurate identification of the SNPs for the standards, buccal swabs were collected from volunteer donors as approved by the Towson University Institutional Review Board. Following DNA extraction and quantitation, each sample was analyzed with the optimized primer sets for each SNP, and the results were used to make phenotypic predictions of eye and skin color using decision tree models for each. The allele(s) present at the six SNPs was confirmed for each donor using Sanger sequencing. PCR HRM assays can be used as preliminary screening tools to determine eye and skin color and are much more cost-effective than MPS. PCR HRM can also be used to determine allele calls when the number of MPS reads at the SNP does not clearly support a determination of heterozygote or homozygote.

#### Reference(s):

1. Hart K.L., Kimura S.L., Mushailov V., Budimlija Z.M., Prinz M., Wurmbach E. Improved eye- and skin-color prediction based on 8 SNPs. *Croat Med J* 2013;54(3):248–56.
2. Kayser M., Schneider P.M. DNA-based prediction of human externally visible characteristics in forensics: Motivations, scientific challenges, and ethical considerations. *Forensic Sci Int Genet* 2009;3(3):154–61.
3. Walsh S., Chaitanya L., Clarisse L., Wirken L., Draus-Barini J., Kovatsi L., et al. Developmental validation of the HIRISplex system: DNA-based eye and hair colour prediction for forensic and anthropological usage. *Forensic Sci Int Genet* 2014;9:150–61.

#### Forensic Science, SNPs, DNA Typing



### **B9 Assessing Potential Inhibitory Effects of Personal Lubricant on the Ability to Detect Biomarkers Consistent With Semen, Saliva, and Vaginal Fluid**

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**Learning Overview:** After attending this presentation, attendees will have gained an understanding of the effects of personal lubricant on the ability to detect and identify protein biomarkers using protein mass spectrometry for the analysis of sexual assault evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an in-depth scientific evaluation describing the adverse effects of lubricant on serological identification and how modified procedures using advanced instrumental techniques can mitigate deleterious effects.

Sexual assault evidence remains one of the most commonly encountered types of evidence. This has prompted extensive research and development in advancing confirmatory serological techniques in order to complement the sensitivity obtained with modern genetic testing. Protein mass spectrometry has been demonstrated as an attractive alternative to traditional testing methodologies, allowing for the confirmatory identification of trace levels of fluid-specific biomarkers. In order to further evaluate the implementation of protein mass spectrometry in relation to sexual assault kit workflow testing, an extensive study has been conducted to evaluate the effects of lubricants on the ability to detect target biomarkers. Lubricants have the potential to inhibit protease activity, displace hydrophobic markers during Solid Phase Extraction (SPE), and suppress ion detection during mass spectrometry analysis.

A previously established assay containing protein biomarkers consistent with seminal fluid (semenogelin 1, semenogelin 2, prostate specific antigen, prostatic acid phosphatase, and epididymal secretory protein), saliva ( $\alpha$ -amylase, statherin, submaxillary gland androgen-regulated protein 3B, and cystatin-SA), and vaginal fluid (small proline rich protein 3, cornulin, neutrophil gelatinase, Ly6/PLAUR, suprabasin, perioplakin, and involucrin) was utilized during the course of this research. Lubricant types assessed included water-based with glycerin, water-based without glycerin, silicon-based, hybrid silicon, and natural oil-based lubricants. Three studies were performed for the completion of this research: (1) determining the ability to detect vaginal fluid biomarkers from vaginal swabs fortified with lubricant; (2) establish the effect of lubricant types on the ability to detect biomarkers of seminal fluid and saliva; and (3) assess the ability to identify biomarkers on pre-lubricated condoms.

Vaginal swabs free of semen or saliva were collected, solubilized, and pooled to create a single-source matrix. The extract volume was normalized and applied to clean cotton-tipped swabs prior to fortification with lubricant and/or seminal fluid and saliva. For the first portion of this research, vaginal swabs were enriched with 1 $\mu$ L, 5 $\mu$ L, or 15 $\mu$ L of lubricant. Samples for the second portion of this study were prepared in a similar manner, with swabs fortified with lubricant and either 1 $\mu$ L of seminal fluid or 10 $\mu$ L of saliva. Last, for the third portion of this study, prepared swabs were moistened and used to sample the external and internal portions of a pre-lubricated condom. This sample set was designed to simulate an authentic vaginal swab recovered from an individual who had been assaulted with a barrier form of contraception.

Swabs were solubilized and protein material was separated from cellular material through centrifugation. Samples were then pre-treated prior to enzymatic digestion using SPE with Waters<sup>®</sup> Oasis<sup>™</sup> HLB 1cc vac cartridges as an additional cleanup to reduce lubricant impact. An Agilent<sup>®</sup> AssayMAP Bravo automation platform was employed for tryptic protein digestion and micro-SPE clean-up. An Agilent<sup>®</sup> 6495 triple quadrupole mass spectrometer coupled to a 1290 series liquid chromatograph was utilized for this study. Data was interpreted according to three criteria: (1) the overall Peak Area Response (PAR) of the target biomarker; (2) biomarker PAR in relation to internal standard; and (3) PAR of digestion control protein. PAR response was dependent on the type of lubricant assessed, with greater effects observed on hydrophobic biomarkers. Water-based lubricants had little-to-no effect on biomarker detection. As hypothesized, increased lubricant volumes resulted in greater PAR loss. Select hydrophobic biomarkers experienced a >80% decrease in PAR with silicon and natural oil lubricants; however, this issue was resolved with additional sample preparation procedures. Furthermore, select hydrophilic biomarkers exhibited ion enhancement in the presence of silicon-based lubricants, the effects of which were also decreased with the additional sample pre-treatment.

In conclusion, lubricant type does affect the ability to accurately identify protein biomarkers. A sample preparation method was developed in order to eliminate a majority of the deleterious effects observed on the consistency of biomarker detection.

#### **Serology, Sexual Assault, Proteomics**





### B10 Developing a Forensically Relevant Single-Cell Interpretation Strategy for Human Identification

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**Learning Overview:** After attending this presentation, attendees will better understand how confounding signal (i.e., allelic drop-out, stutter, and allelic drop-in) adversely affects mixture interpretation of bulk-processed samples and how the characterization of signal garnered from single cells is an important step toward the development of single-cell inference systems.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the potential of single-cell systems to improve mixture interpretation.

Biological evidence submitted to the forensic DNA laboratory contains cells from an unknown number of contributors in unknown proportions, resulting in interleaved mixture profiles, which are difficult to interpret, affecting match statistics or inclusion/exclusion decisions reported to the trier-of-fact.

Given the complexities associated with bulk-mixture interpretation, recent efforts have focused on introducing single-cell systems into the forensic laboratory in order to de-convolve the mixture signal by separating cells at the front end of processing. However, low-template and single-cell signal are often obfuscated by the presence of elevated stutter; false negative detection of alleles, known as allelic drop-out; and false positive detection of alleles, commonly referred to as allelic drop-in.

In this study, 1,845 single-cell profiles were analyzed, 556 of which were acquired from single-source, single-cell samples (laboratory conditions: Globalfiler™ amplification, 30 Polymerase Chain Reaction (PCR) cycles, 25-second injection on ABI® 3500 Genetic Analyzer). The data from all 556 samples was evaluated to determine the distributions associated with allelic drop-out, stutter, and allelic drop-in. The data was statistically assessed to determine whether confounding signal observed in the Electropherograms (EPGs) of single cells was significantly different from artifactual signal acquired from bulk-processed samples. The results demonstrate that, in contrast to bulk-processed samples, allelic drop-out is likely cell dependent. Stutter was again found to be locus dependent. In addition, the propensity of single-cell samples to exhibit elevated stutter ratios was observed. Notably, the stutter percentages for the single-cell samples ranged from 0% to 241% as predicted by the stochastic model described by Duffy et al.<sup>1</sup> Further, the frequency of allelic drop-in was consistent with that of bulk-processed samples (i.e., 0.04%) and cell independent, suggesting that its detection is mainly driven by laboratory parameters and conditions rather than sample condition or total DNA concentration. The presence of cell-dependent drop-out rates and high levels of stutter suggest that if a full evaluation of multiple single-cell signal is desired, new probabilistic constructs that do not rely on assumptions of cell-independence for drop-out are likely required. Since probabilistic inference relies heavily on models that adequately represent the system in question, this study forms the foundation from which these inference systems can be developed.

To further explore the viability of single-cell pipelines for forensic DNA casework, a protocol was developed to desorb buccal cells from cotton-tipped applicators. To measure its efficacy, hemocytometry was used to determine the percent of cells recovered. The 52% recovery of buccal cells was consistent with previous work wherein 54% of DNA was recovered from cotton swabs.<sup>2</sup> These results demonstrate that a single-cell strategy for forensic mixture interpretation is a viable alternative to bulk-mixture interpretation strategies that can lead to signal that is too low or highly masked by other contributors in the DNA mixture.

*This project was supported by the Jan S. Bashinski Criminalistics Graduate Thesis Grant from the Forensic Sciences Foundation.*

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#### Single-Cell, Stutter Artifact, Allelic Drop-Out



### B11 High-Throughput and Simultaneous Analysis of 12 Cannabinoids in Hemp Oil Using Liquid Chromatography With Ultraviolet (LC-UV) Detection

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**Learning Overview:** After attending this presentation, attendees will better understand the strategy to achieve high-throughput and simultaneous analysis of cannabinoids and appreciate a validated LC-UV method for the analysis of 12 cannabinoids in hemp oil.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by first introducing three fast LC separations of 12 cannabinoids that can be used with either UV or Mass Spectrometric (MS) detection. This presentation will further impact the forensic science community by introducing a validated LC-UV method for high-throughput and simultaneous analysis of 12 cannabinoids in hemp oil, which can be routinely used by cannabis testing labs.

In recent years, the use of products of *Cannabis sativa* L. for medicinal purposes has been in a rapid growth, although their preparation procedure has not been clearly standardized and their quality has not been well regulated. To analyze the therapeutic components (i.e., cannabinoids) in products of *Cannabis sativa* L., LC-UV has been frequently used because LC-UV is commonly available and usually appropriate for routine analysis by the cannabis growers and commercial suppliers. In the literature, a few validated LC-UV methods have been described. However, so far, all validated LC-UV methods only focused on the quantification of 11 or fewer cannabinoids. Therefore, a method able to simultaneously analyze more cannabinoids in a shorter run time is still in high demand because more and more cannabinoids have been isolated and many of them have shown medicinal properties.

In this study, the LC separation of 12 cannabinoids, namely Cannabichromene (CBC), Cannabidiolic Acid (CBDA), Cannabidiol (CBD), Cannabidivarinic Acid (CBDVA), Cannabidivarin (CBDV), Cannabigerolic Acid (CBGA), Cannabigerol (CBG), Cannabinol (CBN), Delta-8 Tetrahydrocannabinol ( $\Delta^8$ -THC), Delta-9 Tetrahydrocannabinolic Acid A ( $\Delta^9$ -THCA A), Delta-9 Tetrahydrocannabinol ( $\Delta^9$ -THC), and Tetrahydrocannabivarin (THCV), has been systematically optimized using a Phenomenex® Luna® Omega 3 $\mu$ m Polar C18 150mm  $\times$  4.6mm column with regard to the effects of the type of organic solvent (i.e., methanol and acetonitrile), the content of the organic solvent, and the pH of the mobile phase. The optimization has resulted in three LC conditions at 1.0mL/minute able to separate the 12 cannabinoids: (1) a mobile phase consisting of water and methanol, both containing 0.1% formic acid (pH 2.69), with a gradient elution at 75% methanol for the first 3 minutes and then linearly increase to 100% methanol at 12.5 minutes; (2) a mobile phase consisting of water and 90% (v/v) acetonitrile in water, both containing 0.1% formic acid and 20mM ammonium formate (pH 3.69), with an isocratic elution at 75% acetonitrile for 14 minutes; and (3) a mobile phase consisting of water and 90% (v/v) acetonitrile in water, both containing 0.03% formic acid and 20mM ammonium formate (pH 4.20), with an isocratic elution at 75% acetonitrile for 14 minutes.

In order to demonstrate the effectiveness of the achieved LC separations, an LC-UV method is further validated for the high-throughput and simultaneous analysis of 12 cannabinoids. The method used the mobile phase at pH 3.69, which resulted in significant improvement in throughput compared to other validated LC-UV methods published so far. The method used flurbiprofen as the internal standard. The linear calibration range of all the cannabinoids were between 0.1 to 25ppm with  $R^2 \geq 0.9993$ . The Limit of Quantitation (LOQ) ( $S/N=10$ ) of the cannabinoids was between 17.8 and 74.2ppb. The validation used a hemp oil containing 3.2wt% CBD and no other cannabinoids, which was reported by the vendor with a certificate of analysis, as the matrix to prepare control samples: the hemp oil was first extracted using Liquid-Liquid Extraction (LLE) with methanol; cannabinoids were then spiked into the extract at both 0.5ppm and 5ppm levels. Afterward, the recovery, precision (%RSD), and accuracy (%Error) of the control samples were assessed, and the results met the requirements by the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025 and American Society for Testing and Materials (ASTM) E2549-14 guidelines.

#### Cannabis, Cannabinoids, LC-UV



## B12 Cannabinoid Content in Commercially Available Oils

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**Learning Overview:** After attending this presentation, attendees will better understand a method used to analyze the Cannabidiol (CBD) content in seven commercially available oils.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a quantitative analysis of commercially available cannabinoid products using Gas Chromatography (GC) and Mass Spectrometry (MS).

CBD is a non-psychoactive component found in *Cannabis sativa* (hemp), typically extracted from the plant via supercritical CO<sub>2</sub> extraction.<sup>1</sup> The Farm Bill of 2018 saw the legalization of CBD derived from hemp with less than 0.3% THC content by dry weight in the United States. CBD can now be purchased in tincture, oil, capsule, topical, and edible forms from local stores, in addition to online. In Florida, the sale of hemp products, which includes CBD oils, was recently legalized with SB 1020. SB 1020 mandates that all CBD products be tested by an independent laboratory and have this analysis available on the product packaging, but many of the samples obtained lacked this information. However, many of these products contain Tetrahydrocannabinol (THC), a schedule I drug under the Drug Enforcement Administration (DEA), which poses the question of how hemp products are regulated. In addition, there is no Food and Drug Administration (FDA) regulation for most of the manufacturers of these products, and no way to know if the manufacturers' claims of THC and CBD concentrations are legitimate. This project looks at seven commercially available CBD tinctures and analyzes the advertised CBD concentration compared to the concentration found via GC analysis.

An extracted solid CBD sample was obtained from Extract Labs™ and determined to be of pure quality by Botanacor, a third-party lab, and in-house via melting point analysis (66.5–67.3°C) and GC-MS. A seven-point calibration curve was made with this standard in a solvent of 9:1 hexane: 2-propanol. Initial quantitation on seven CBD tinctures was performed based on this curve. Tinctures were obtained from Tampa Bay, FL, area shops and online. Matrices were Extra Virgin Olive Oil (EVOO), glycerin, and Medium-Chain Triglycerides (MCT) (coconut oil). The glycerin samples were diluted in 2-propanol, and EVOO and MCT were diluted in the 9:1 hexane:2-propanol solution. The samples were then run on Thermo Scientific™ Trace GC Ultra GC/Flame Ionization Detector (FID) with dichloromethane as the wash solvent to minimize carryover. After quantitation, the calculated concentrations of each tincture were compared to the advertised concentration. The lab analysis data was also reviewed, if available from the manufacturer of the tincture.

Initial data suggest that five of the samples have a lower CBD concentration than advertised, and two of the samples have a higher CBD concentration than advertised. It is also suggested that some of the samples also contain THC. The initial data suggests that the manufacturers' advertisement of THC-free CBD tinctures is not accurate, both with the THC-free claim and the concentration advertised. Current work is focused on revising the quantitation method to better assess precision and robustness. Additionally, a separate MS-based method is being developed to quantitate the trace amounts of THC in the oils.

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### Cannabidiol, Cannabinoids, GC/FID



### **B13 The Detection and Identification of Synthetic Cathinones by Portable Nano-Liquid Chromatography (Nano-LC) With Dual Wavelength Ultraviolet (UV) Detection**

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**Learning Overview:** After attending this presentation, attendees will understand how different synthetic cathinones can be screened for and identified by portable nano-LC with dual wavelength UV detection.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the first portable nano-LC with UV detection that can be used in the field or laboratory for the screening or identification of emerging drugs, specifically synthetic cathinones. This is a valuable technique because, unlike other portable devices, it is both lightweight and highly amenable to mixtures. In terms of identification, the proposed technology presents a low-cost option to meeting the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) guidelines.

Emerging drugs have been created to avoid controlled substances laws. These drugs, also known as “designer drugs,” are created by slightly altering the chemical structure of a current controlled substance. Since these drugs are not regulated by controlled substances laws, they can be purchased over the internet and in local smoke shops. Synthetic cathinones are an example of designer drugs. They are a group of amphetamine compounds derived from the active stimulant (cathinone) of the khat plant. These drugs imitate the effects of controlled stimulant drugs, such as methamphetamine, Methylenedioxymethamphetamine (MDMA), and cocaine. The desirable effects of synthetic cathinones felt by users include increased alertness, euphoria, increased sociability, and improved attention span.

The most commonly used field test for the preliminary identification of seized drugs are color tests, which lack specificity. Portable instruments employed, including Raman, Infrared (IR) spectroscopy, Ion Mobility Spectrometry (IMS), and Gas Chromatography/Mass Spectrometry (GC/MS), can be used for the on-site identification of seized drugs but have limitations in the identification of synthetic cathinones. Portable Raman and IR spectroscopy are problematic for the identification of mixtures. Portable IMS, which can separate mixtures based on their mobility in a buffer gas, lacks specificity. GC/MS can both separate and identify mixtures of seized drugs, but is limited for the identification of synthetic cathinones due to indistinguishable mass spectra and retention times of certain positional isomers.

SWGDRUG guidelines specify for drug identification that when a category A test is unavailable, alternatively acceptable are two procedures from category B and a third from category C. The category B tests include two retention times using uncorrelated separation systems, while the category C is of lower specificity.

The portable nano-LC instrument employed in this investigation is the first of its kind; weighing only 16 pounds, it can easily be transported between the laboratory and the field. The instrument’s software can be run on a laptop or tablet connected through built-in Wi-Fi and has a rechargeable battery that can be used for 10+ hours. Because of its size, the instrument uses a fraction of the solvents and produces a fraction of the waste of conventional laboratory LC systems. The nano-LC can be equipped with different tandem columns in series with on-column LED UV detection at two wavelengths. This instrument is ideal for the screening of seized drugs because it can give two uncorrelated retention times and peak area or peak height ratios (dependent on absorbance ratios) that are proportional to the drug’s extinction coefficient at both wavelengths. In addition, based on the multitude of identification points, it could meet SWGDRUG guidelines for drug identification. The present study employs two capillary columns in series (C8 and biphenyl) and dual UV detectors (255nm and 275nm) for the screening and/or identification of different synthetic cathinones, including in the presence of adulterants. These drugs are identified by the ratios of the relative retention times for both capillaries and by their peak height or peak area ratios determined by dual wavelength detection.

#### **Portable Nano-LC, Emerging Drugs, Synthetic Cathinones**



### B14 Retrofitting Massively Parallel Sequencing (MPS) for HLA-DQA1 and Polymarker (PM) in Forensic Casework

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**Learning Overview:** After attending this presentation, attendees will be able to understand the potential use of MPS in forensic casework where no original biological evidence remains, but HLA-DQA1 and PM forensic analysis results are present.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an alternative method to approach cold cases and cases in which biological evidence, originally processed with the AmpliType® PM+DQA1 PCR Amplification and Typing Kit, is no longer available.

The objective of this research is to retrofit MPS to establish the genetic profile of an alleged suspect in a cold case, where the sole remaining evidence for comparison are results from the AmpliType® PM+DQA1 Kit. The AmpliType® PM+DQA1 Kit simultaneously amplified six loci: HLA Class II Histocompatibility Antigen gene coding for the DQ Alpha 1 Chain (HLA-DQA1), Low-Density Lipoprotein Receptor (LDLR), Glycophorin A (GYPA), Hemoglobin G Gammaglobin (HBGG), Group-specific Component vitamin D Binding Protein (GC), and D7S8.<sup>1</sup> The methodology was based on a reverse dot blot assay, which utilized an allele-specific oligonucleotide probe, immobilized on a nylon membrane, to hybridize with the complementary DNA sequence.<sup>1</sup> Following hybridization, a colorimetric assay indicated the presence of the corresponding allele.<sup>1</sup> The AmpliType® PM+DQA1 PCR Kit was one of the first Polymerase Chain Reaction (PCR) -based DNA typing kits. It emerged in the early 1990s as a popular technique that provided improved sensitivity compared to Restriction Fragment Length Polymorphism (RFLP) assays, although it had a lower power of discrimination. The assay was soon replaced by Short Tandem Repeat (STR) -based assays in the mid to late 1990s. This presentation aims to communicate the development of an MPS assay to type the six original loci and depict the results of preliminary testing on reference samples.

Primer and probe sequences were obtained from the literature.<sup>2,3</sup> Primers were synthesized for compatibility with the Ion AmpliSeq™ targeted sequencing technology in collaboration with Thermo Fisher Scientific™. Additional primers were designed for redundancy. Initially, samples of known genotypes were amplified in a singleplex reaction using conventional PCR with AmpliTaq Gold® DNA Polymerase. Successful amplification was verified utilizing agarose gel electrophoresis. PCR products were subsequently pooled and processed with the Ion AmpliSeq™ manual library preparation protocol. The barcoded samples were later templated and sequenced on the Ion Chef™ and Ion S5™ platform using the Ion S5™ Precision ID Chef & Sequencing Kit. In parallel, primers were pooled and samples were processed with the automated library preparation Precision ID DL8 kit and sequenced as described above. The sequenced samples were analyzed using Integrated Genome Viewer (IGV) software through localization of probe sequences for each marker to determine the genotype of each sample and compared to known references for verification. The most challenging region to type was GYPA due to the similarities between GYPA, Glycophorin B (GYPB), and Glycophorin E (GYPE). GYPA, GYPB, and GYPE are all sialoglycoproteins found on the human erythrocyte membrane, and they help determine the MNS blood groups. There is 95% homology among them.<sup>4</sup>

Preliminary results indicate that this MPS-based assay is able to effectively type the loci targeted in the original AmpliType® PM+DQA1 PCR Amplification and Typing Kit. Results from the manual library preparation are consistent with the results obtained utilizing an automated approach. As expected, sequencing the HLA-DQA1 region is more discriminating than the original assay as it allows for detection of microvariants indiscernible to the probe hybridization-based methodology.

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#### HLA-DQA1 Polymarker, Massively Parallel Sequencing, Cold Case



### **B15 A Method Development for Analyzing 17 Benzodiazepines and Metabolites Used in Crimes Using Solid Phase Extraction-Tandem Mass Spectrometry**

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**Learning Overview:** The goal of this presentation is to explain the results of a research project on developing an analytical method for detection, identification, and quantification of drugs used in crimes, at very low concentrations, using Solid Phase Extraction coupled to Liquid Chromatography/Tandem Mass Spectrometry (SPE-LC/MS/MS), which can be used in analyzing these drugs in biological samples, such as urine and blood.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a newly developed analytical method for detection, identification, and quantification of drugs used in crimes at very low concentrations in biological samples such as urine and blood. Victims of Drug Facilitated Crimes (DFCs) often report the crime hours or even days after they have happened, which adds to the challenge of detecting the drugs in the biological samples by the currently used analytical methods in forensic toxicology laboratories. The developed method can help detect the drugs at lower concentrations than possible with the current methods, which can extend the time the drugs can be detected in victims' urine or blood samples, giving them a better chance to prove the case.

Benzodiazepines are a class of antianxiety drugs, including drugs such as flunitrazepam, alprazolam, and diazepam. They are also known as predator or date-rape drugs used in DFCs—crimes that include robbery, the maltreatment of the elderly and children, as well as rape and other sexual assaults. Identification of these drugs, or their metabolites, in biological specimens such as the urine, blood, saliva, and hair of victims is commonly proof of exposure to the drug.

Like other psychoactive drugs used in DFCs, benzodiazepines are highly potent and have short half-lives. The combination of potency and short half-life makes the time window for when the drug is still detectable in biological samples very small. Victims of DFCs usually experience short-term memory loss and often report the crime days after they have happened, which adds to the challenge of detecting the drugs in the biological samples by the currently used analytical methods in forensic toxicology laboratories. The goal of the current project is to develop a method for detection, identification, and quantification of 17 benzodiazepines at sub-ppb concentrations in aqueous solutions and apply it to the analysis of these drugs and their metabolites in biological samples such as urine and blood.

In this study, an SPE-LC/MS/MS method was successfully optimized. A Supel™-Select HLB 54183-U 200mg cartridge was selected for SPE. Washing and conditioning of the packing was completed using 12mL of methanol and 2mL of ultra-pure water, respectively. After sample introduction, the analytes of interest were eluted using 5mL of a 50:50 mixture of methanol and acetonitrile. The elutant was evaporated using rotary evaporation and, upon dryness, was reconstituted using 1mL of 0.01M ammonium acetate. The reconstituted sample was then subjected to LC using C18 HPLC column (particle size: 1.9µm and length: 20cm) with gradient elution with a mixture of acetonitrile and 0.01M ammonium acetate buffer solution. The elution started with 100% 0.01-M ammonium acetate and was gradually decreased to 10%, while acetonitrile was increased to 90% and held for half a minute. Then 0.01-M ammonium acetate was increased back to 100% and held for four minutes. For MS detection, a Selected Ion Monitoring (SIM) method was started at minute 3.00 of analysis until minute 8.00. Using the optimized SPE and LC/MS/MS method, this study determined the Limits Of Detection (LODs) for 17 analytes to be in the range of 0.01 to 1.8ng/ml. The optimized method was applied to analyze the drugs in the spiked urine samples and will be applied to spiked blood samples.

#### **Benzodiazepines, Drug Facilitated Crimes (DFCs), SPE-LC/MS/MS**



### B16 “Free Range” Gunshot Primer Residue: A Study on Multiple Transfers of Gunshot Primer Residue

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the number of times Gunshot primer Residue (GSR) can transfer from one surface to the next.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing practitioners insight into the dynamics of GSR particle transfers from one surface to a second surface to a third surface. This presentation describes the methods used to test this theory of transfer of GSR.

GSR is produced by a firearm when it is discharged. The primer for centerfire cartridges is mainly composed of lead styphnate, barium nitrate, and antimony sulfide. The residue from the primer explosion escapes from openings in the gun and can be deposited on a person’s hands and clothing. These particles can be collected and analyzed using automated scanning electron microscopy/energy dispersive X-ray spectroscopy. Characteristic GSR particles have a molten appearance and contain barium, antimony, and lead. There have been several studies that examine the transfer of GSR to the interior of police cars from a person who has gunshot residue on their person. There have not been studies conducted that try to determine the number of times GSR particles could transfer from one surface to another.

This presentation will detail the results of two studies on the potential transfer of GSR. The first study involves the transfer of GSR from a GSR-contaminated area to a clean subject who enters the area, then subsequently transfers the gunshot residue particles to other surfaces outside the contaminated area. One of the analysts of the Trace Evidence section of the laboratory attended firearm familiarization training given by the Firearms and Toolmarks section of the laboratory. This analyst does not handle firearms at all. At the end of the training, the analyst was asked to stub her clothing, her cubicle chair, the driver seat of her car, and any chairs that she might have sat in at her home in the same clothing she had worn to the training.

The second study deals with the potential transfer of GSR from a victim’s clothing to a person’s hands that touch the victim. The Firearms and Toolmarks section re-created a shooting victim’s clothing. An analyst who had no GSR on their hands handled the shooting victim’s clothing. Their hands were then stubbed to analyze for GSR.

All of the above stubs were analyzed using scanning electron microscopy energy dispersing X-ray spectroscopy instrumentation using standard laboratory procedures for the analysis of GSR.

This research takes a novel approach by investigating the likelihood of multiple transfers of GSR particles between surfaces.

#### **Gunshot Primer Residue, Scanning Electron Microscopy, Locard’s Theory**



### **B17 Ambient Ionization Mass Spectrometry (AMS): A New Forensic Tool for Adhesive Tape Evidence Discrimination**

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**Learning Overview:** After attending this presentation, attendees will be better informed about AMS techniques for the analysis of tape evidence. In particular, the methodology will explore analyzing different types of adhesive tapes to identify discriminating mass spectral properties. AMS techniques were utilized in new ways to aid forensic investigations in exploiting novel evidence items, such as tape.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an additional technique for analyzing forensic adhesive tape evidence. This technique was optimized to distinguish the manufacturer of the tape samples. This new technique is able to provide data in real time that has the potential to expedite a forensic investigation. Additionally, this technique could be optimized for other sample types. The optimization of the different types of AMS sources could lead to a wider applicability of this methodology as a tool in discriminating other evidence types commonly encountered in the crime scene.

Adhesive tapes are often found at crime scenes, and these tapes supply numerous investigative leads, if treated properly. Trace evidence (hair, fibers, etc.) are often found on tapes, along with DNA and fingerprints. Physical tear matches can also be performed to link a piece of tape to the original roll it came from. There is also one other way that forensic professionals analyze adhesive tapes, and that is via chemical analysis. Chemical analysis of adhesive tapes is where the adhesive and/or backside layer of the tape are analyzed to get its chemical composition. The chemical information is used to differentiate the tapes by type and/or brand, which is important because it allows investigators to make connections between suspects and the tape evidence. Several chemical analysis techniques, including Fourier Transform Infrared (FTIR), Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS), Isotope Ratio Mass Spectroscopy (IRMS), Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS), Nuclear Magnetic Resonance (NMR), and Energy-Dispersive X-Ray Spectroscopy (EDX), have been shown to yield different accuracy in distinguishing tape brands, but, while they offer several advantages, they may require intensive sample preparation, complex instrumentation with limited accessibility, or are destructive in nature. AMS is a relatively new, easy-to-use, cost-effective, and portable technique that allows direct desorption/ionization from sample substrates with little-to-no sample preparation to yield chemical analysis in real-time.

The purpose of this study was to develop and evaluate different AMS approaches, such as direct desorption Flowing Atmospheric Pressure Afterglow (FAPA), and Laser-assisted Desorption (LD) AMS for analyses capabilities of adhesive tapes. With these different approaches, multiple different tape types were analyzed, including silver duct tape and black electrical tape. There were numerous manufacturers of each tape tested to be able to distinguish and categorize the types of tape, and the brands of each tape. Data were analyzed using Principle Component Analysis (PCA) and the Partial Least Squares (PLS) method in order to find the covariance and correlations that lead to the discrimination of tapes by manufacturer. This technique captured an array of structural information for each tape under study, thereby highlighting promising results of ambient ionization techniques as a forensic tool.

#### **Adhesive Tape, Ambient Ionization, Manufacturer**





### B18 Supplementary Viewing Techniques of Gunshot Residue (GSR) Utilizing Infrared (IR) and Fluorescence

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**Learning Overview:** The goal of this presentation is to inform attendees about the use of IR and fluorescent photography as a method for visualizing GSR on dark, patterned, or contaminated clothing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating techniques capable of visualizing GSR on normally difficult-to-work-with evidence in a rapid and non-destructive manner.

While muzzle-to-target analysis is a crucial component of forensic investigation into firearms-related incidents, a number of obstacles can impede the ability of traditional techniques to observe depositions of GSR at an acceptable level. Microscopic examination for the purpose of documenting GSR deposition patterns is difficult to perform on evidence comprised of darker or patterned fabrics, and environmental contaminants can affect both the microscopic examinations and follow-up chemical testing. For these reasons, development of a supplementary non-destructive technique for GSR visualization is desirable.

Previous studies have demonstrated that GSRs possess both IR and fluorescent properties, allowing for the visualization of deposition patterns on a variety of substrates, though no definitive methodology has currently been established. This study evaluates the ability of the Foster and Freeman Crime-lite® 82S series of light sources, as well as the Foster and Freeman DCS® 5 workstation, for the purpose of visualizing GSRs across a number of variables.

In order to establish that GSR deposition patterns observed utilizing IR mimicked expected results derived from more easily observed lighter fabrics, dark fabrics were shot in triplicate over a selection of fixed distances and calibers. These results were compared to a light fabric control shot with the related caliber at each distance. Testing was then expanded to incorporate a variety of ammunition manufacturers and fabric types to discern the effects, if any, each of these variables had on visualization with IR. Obtained samples were also subjected to fluorescent testing utilizing a green light source (490–560nm) coupled with a deep-red filter (571nm) in order to serve as an additional method for GSR visualization and as a comparison point for obtained IR images. Final testing involved the application of a selection of commonly encountered environmental contaminants to fired targets in order to document their potential interference in the visualization process.

The results of this study indicate that visualization of GSR on dark fabrics utilizing IR in ideal conditions is widely successful across most tested calibers and manufacturers, while fabric composition, weave, and pattern differentially affected IR visualization. Green fluorescent visualization was tested with varying outcomes observed across multiple calibers, manufacturers, and target materials. Contaminants were also demonstrated to have varied effects on visualization as certain environmental contaminants were able to be eliminated nearly completely while others completely obscured GSR patterns. Future avenues for investigation can involve testing the effects of different fluorescent wavelengths and additional contaminants or utilize a more comprehensive list of distinct ammunition types and manufacturers.

#### Gunshot Residue, Infrared, Fluorescence



### B19 The Development of a Rapid, Reliable Detection Method for Synthetic Cathinones

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**Learning Overview:** After attending this presentation, attendees will understand the principles of Surface-Enhanced Raman Spectroscopy (SERS), how the application of SERS is used to rapidly detect synthetic cathinones, and how selected bands can be utilized as markers of these substances.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the application of SERS to the detection of synthetic cathinones, such as 3,4-methylenedioxy-5-methylethcathinone and 4-methylethcathinone.

Effectively examining synthetic cathinones will contribute to the prevention of their proliferation and to the assistance of victims suffering from overdose, irreversible health damage, or even death. Improvements in the control of these compounds will also aid toxicologists and forensic scientists in detecting and limiting their effect on society. Furthermore, being able to detect these synthetic drugs will provide critical assistance in understanding the effects of these substances on the human body.

Currently, countless structural variations of new synthetic cathinones (“bath salts”) appear in drug markets. However, the research into their specific toxicological effects in humans is lacking. Manufacturers synthesize these new synthetic cathinones in various ways, and many of these drugs are sold as mixtures containing additives. Variations in the purity of these drugs and, thus, the presence of additives at unknown concentrations, can cause overdoses. These mixtures cause problems for both policing and health agencies. Therefore, there is a need for a screening method that can assist authorities in identifying these drugs in a more rapid, sensitive, and inexpensive way.

Since the drugs studied in this proposal are structurally similar, SERS is uniquely suited as a screening procedure. Due to its molecular specificity and sensitivity, SERS constitutes an ideal platform to detect and differentiate synthetic cathinone analogs. SERS utilizes nanometallic surfaces to amplify the Raman signal of analytes, providing qualitative and quantitative information. The oscillations of the conduction electrons of these nanometallic structures causes the accumulation of photonic energy and results in Localized Surface Plasmon Resonances (LSPRs). LSPRs are responsible for the signal enhancement that allow adsorbed molecules to be detected, even at low concentration levels. LSPRs can also produce signal enhancements such that single molecule signals can be detected. This provides a flexible method to detect newly developed compounds and keep them off the streets.

The synthetic cathinones 3,4-methylenedioxy-5-methylethcathinone and 4-methylethcathinone have been analyzed with both traditional Raman spectroscopy and SERS. The methods for their detection have been optimized by varying pH, incubation time, nanoparticle size and composition, aggregating agents, and laser wavelength. Density Functional Theory (DFT) calculations have been used to assign the vibrational modes of major bands for each of the selected synthetic cathinones, providing a sound spectroscopic interpretation of the experimental Raman and SERS spectra. Further validation of this work will be completed with the ultimate aim of detecting these drugs and their analogs in biological matrices, starting with oral fluid.

#### Synthetic Cathinones, Surface-Enhanced Raman Spectroscopy, Density Functional Theory



### B20 A Validation Study of the Synthetic Cannabinoid 5-Fluoro PB-22

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**Learning Overview:** The goal of this presentation is to educate attendees on the growing problem of synthetic cannabinoids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how dangerous and prominent synthetic cannabinoids are becoming as well as by providing a method by which 5-fluoro PB-22 can be identified in samples using Gas Chromatography/Mass Spectrometry (GC/MS) analysis.

**Background/Introduction:** The first synthetic cannabinoid was developed in the late 1980s by chemist John W. Huffman in an effort to understand the two cannabinoid receptors (CB<sub>1</sub> and CB<sub>2</sub>). The cannabinoid receptors are not only involved in the high experienced by marijuana users, but also in the control of appetite, pain, and sleep. While the development of synthetic cannabinoids led to a greater understanding of the cannabinoid receptors, it also precipitated an entirely new drug market. Clandestine chemists hijacked scientific research, using published scientific articles to synthesize cannabinoids for distribution in the gray market. Products containing synthetic cannabinoids were marketed as Spice, herbal blends, or incense. The products are labeled “to be used as incense” and “not for human consumption” in an effort to circumvent drug laws and regulations. Spice products can contain more than a dozen synthetic cannabinoids of varying concentrations.

**Objective:** The focus of this project is to develop and validate a method for the analysis of the synthetic cannabinoid 5-fluoro PB-22, which first appeared in 2014. 5-fluoro PB-22 has high affinities for both cannabinoid receptors, which is significant because  $\Delta^9$ -Tetrahydrocannabinol (THC) is only a partial agonist for both of the cannabinoid receptors.

**Methods:** This validation includes studies determining the Limit Of Detection (LOD), the accuracy of identification, and specificity of the assay. In addition, a GC/MS program has been developed to differentiate between 5-fluoro PB-22 and 13 of its isomers. The isomers of 5-fluoro PB-22 that were examined included five hydroxyquinoline isomers, five hydroxyisoquinoline isomers, and three N-(fluoropentyl) isomers. Finally, casework was simulated using previously confiscated samples submitted by law enforcement to the Alabama Department of Forensic Sciences.

**Results:** The LOD for 5-fluoro PB-22 is 20 $\mu$ g/mL using the developed method. The method provided reproducible results over the course of three different days. Two different isomers, 5-fluoro PB-22 4-hydroxyquinoline isomer and 5-fluoro PB-22 5-hydroxyquinoline isomer, are the only isomers that resulted in interference with the target drug. 5-fluoro PB-22 5-hydroxyquinoline interfered with library matches as well as ion ratios. 5-fluoro PB-22 4-hydroxyquinoline isomer had a retention time interference with the target drug. Additionally, three additional synthetic cannabinoids (JWH 081, AKB48 N-(4-fluorobenzyl) analog, and 5-fluoro THJ) were also found to interfere with the library matches to the target drug as well as ion ratios. The method was able to identify 5-fluoro PB-22 in ten simulated case samples with concentrations of the drug ranging from 75 $\mu$ g/mL to 500 $\mu$ g/mL.

**Conclusion/Discussion:** The LOD falls within an acceptable range for the purpose of the assay. The developed method has provided reliable and reproducible results in the analysis of the target drug. The method has also provided reliable results in identifying the drug in simulated case samples. Of the 145 synthetic cannabinoids tested, two isomers of 5-fluoro PB-22 have caused interference with the analyte. Currently, separation of 5-fluoro PB-22 from the two interfering isomers has not been achieved, but work is ongoing.

#### Synthetic Cannabinoid, Clandestine Chemistry, Spice

## B21 Metabolism of Furanylfentanyl in Fresh Human Hepatocytes: Detection and Confirmation of Furan Ring-Opened Carboxylic Acid Metabolite

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**Learning Overview:** After attending this presentation, attendees will have gained a deeper knowledge regarding the metabolism of furanylfentanyl, an analog of fentanyl that has a furan-2-carbonyl group instead of the propionyl group found in fentanyl. Furanylfentanyl has been available in the United States and Europe since at least 2015 and has been detected in more than 18 countries.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the metabolic pathways of furanylfentanyl definitively determined using authentic standards of its metabolites.

In the present study, the metabolism of furanylfentanyl was investigated using fresh human hepatocytes. Four metabolites of furanylfentanyl were definitively identified in the culture medium of hepatocytes incubated with furanylfentanyl by comparing the analytical data with chemically synthesized authentic standards.

**Methods:** The authentic standards of furanylfentanyl and its five putative metabolites (nor-furanylfentanyl, 4'-hydroxy-furanylfentanyl,  $\beta$ -hydroxy-furanylfentanyl, 4'-hydroxy-3'-methoxy-furanylfentanyl, and furan ring-opened carboxylic acid metabolite) were synthesized in the laboratory. Fresh human hepatocytes (seeded in a 24-well plate at  $2.1 \times 10^5$  cells/cm<sup>2</sup>) were incubated with 10 $\mu$ M furanylfentanyl at 37°C and 5% CO<sub>2</sub>, and the culture medium was sampled 48h after addition of the drug. The medium was treated with  $\beta$ -glucuronidase/aryl sulfatase, followed by deproteinization by the addition of acetonitrile. After centrifugation, the supernatant was dried under a stream of nitrogen and reconstituted with the initial mobile phase used in liquid chromatography. Metabolites in the samples were analyzed via liquid chromatography-triple stage quadrupole mass spectrometry.

**Results:** 4'-Hydroxy-furanylfentanyl ([M+H]<sup>+</sup>;  $m/z$  391),  $\beta$ -hydroxy-furanylfentanyl ( $m/z$  391), 4'-hydroxy-3'-methoxy-furanylfentanyl ( $m/z$  421), and furan ring-opened carboxylic acid metabolites ( $m/z$  409) were detected on the Extracted Ion Chromatograms (EICs) of their protonated molecules. Furan ring-opened carboxylic acid metabolite was considered the main metabolite of furanylfentanyl owing to its extremely strong peak intensity. The other three metabolite peaks were minimally detected in the EICs. In the case of fentanyl, the desphenethylated metabolite, nor-fentanyl, was the main metabolite found *in vivo* and *in vitro* in the previous study; however, the desphenethylated metabolite of furanylfentanyl, nor-furanylfentanyl, was not detected in the culture media at all. To date, two studies on the metabolism of furanylfentanyl have been published.<sup>1,2</sup> Both studies reported that the dihydrodiol metabolite ([M+H]<sup>+</sup>;  $m/z$  409, identical to the furan ring-opened carboxylic acid metabolite) is formed from furanylfentanyl and the presence of the furan ring-opened carboxylic acid metabolite was ruled out. However, the dihydrodiol metabolite was not identified with authentic standards in those studies. In the present study, the metabolite with a molecular weight of 408 ([M+H]<sup>+</sup>;  $m/z$  409) was definitively identified as a furan ring-opened carboxylic acid metabolite by comparing the analytical data with authentic standards. Several studies have reported the formation of furan ring-opened carboxylic acid metabolites. For example, loop diuretic furosemide and  $\alpha_1$ -blocker prazosin are known to form furan ring-opened carboxylic acid metabolites. Therefore, these facts support the results obtained in this study.

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2. Watanabe S., Vikingsson S., Roman M., Green H., Kronstrand R., Wohlfarth A. *In vitro* and *in vivo* metabolite identification studies for the new synthetic opioids acetylfentanyl, acrylfentanyl, furanylfentanyl, and 4-fluoro-isobutyrylfentanyl. *AAPS J* 2017;19:1102-22.

### Furanylfentanyl, Metabolism, Hepatocyte



### **B22 An Analysis of Smokeless Powders, Smokeless Powder Residues, and Pyrolysis Products by Gas Chromatography/Mass Spectrometry (GC/MS)**

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**Learning Overview:** After attending this presentation, attendees will understand the analysis and classification of smokeless powders and post-burn smokeless powder residues, as well as how the pyrolysis products generated in the residues are related to the compounds present in the unburned samples through GC/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how the pyrolysis products generated through the burning of smokeless powders may be related to the intact smokeless powder. The research presented herein may aid in providing a link between a suspect and a crime scene and may be applied to analyses such as organic gunshot residue analysis or analysis of debris after an explosive event.

Following an explosive event, such as the detonation of an improvised explosive device or discharge of a firearm, intact or partially burned smokeless powder particles and burned smokeless powder residues may be recovered. Therefore, to best simulate possible real-world evidence, intact and burned samples were analyzed. Intact smokeless powders were extracted for GC/MS analysis through a simple extraction procedure set forth by the National Center for Forensic Science, using Dichloromethane (DCM) and an internal standard of undecane. Smokeless powders were burned to generate smokeless powder residues. The residues were extracted from foil with approximately 2mL DCM, followed by evaporation and reconstitution in 250 $\mu$ L DCM with an *n*-tetracosane internal standard. Additionally, standards of common smokeless powder compounds, such as nitroglycerin and ethyl centralite, were burned in the presence of Nitrocellulose (NC) to generate pyrolysis products for each compound individually that could be compared with the residue of actual smokeless powder samples. An individual sample of NC pyrolysis products alone was also generated to determine pyrolysis products of NC that may be observed in all samples. The pyrolysis product residues were extracted in DCM. All extracts were analyzed via GC/MS to allow for identification of smokeless powder compounds and pyrolysis products present from each compound and in the smokeless powder residue. GC/MS parameters were optimized for each sample type prior to analysis.

Organic compounds of interest were easily identified in smokeless powders, smokeless powder residues, and individual compound pyrolysis products. The residue composition may then be related to the intact smokeless powder composition. Pyrolysis product peaks were identified in the smokeless powder residue extracts using the data gathered from the individual compound pyrolysis products. Additionally, Pearson correlation coefficients were calculated between the smokeless powders and smokeless powder residues, as well as the smokeless powder residues and pyrolysis samples to determine the similarity in their resulting patterns.

GC/MS is a standard method for analysis that is widely available to investigators. Thus, analysis and characterization of smokeless powders and smokeless powder residues via GC/MS may be beneficial to the forensic community. Additionally, the ability to characterize smokeless powder residues and relate the residues to an original powder composition may be helpful in determining the brand or origin of a suspect sample and provide a link for investigators between a suspect and crime scene.

#### **Smokeless Powder, Explosives, Organic Gunshot Residue**



### B23 An Analysis of Test Exemplar Techniques in Relation to Footwear Examinations

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**Learning Overview:** This presentation will determine the best practices for obtaining the most detail in the test exemplars. This in turn will lead to developing potential for more accurate and detailed footwear impression examinations. The main objective of this study was to compare one technique to another to determine the one with the most reliability.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that acquired characteristics observed in the outsole of four pairs (eight shoes) of athletic shoes can be used to differentiate wear in a common sole design and will provide the best practices for obtaining the most detail in the test exemplars. This in turn will lead to developing potential for more accurate and detailed footwear impression examinations.

The Adidas® Superstar athletic shoes (size 4½) were worn by the same female subject (50 years old) over a period long enough to cause noticeable and unique wear, cuts, and stone-hold characteristics on the outsoles. These randomly acquired characteristics were studied and compared to the characteristics observed in the scanned outsole of each shoe. After collecting the shoes, six techniques were used for obtaining test exemplars. These were: Kiwi® polish, magnetic powder, TreadPrint®, Speedball® Ink and Inkless Shoe Print, WD40® with magnetic powder, and bichromatic powder. The outsoles of each shoe were then photographed and documented after using each of the six techniques. They were then re-examined to locate the visible randomly acquired characteristics.

A total of 48 test exemplars were created and the collected data statistically analyzed using chi-square and the Analysis of Variance (ANOVA) test. Significance was determined by p values <0.05. A comparison of these randomly acquired characteristics revealed that, although the numbers varied, the results were consistent. They also indicated that any of the methods were as good as the others for defining the acquired characteristics developed in the similar sole pattern. The resulting exemplars were suitable for footwear examination.

Footwear examiners will use any and all information available to provide a complete examination of the evidence presented to them. Photographic documentation of the footwear impression evidence on scene alongside the documentation of the outsole of the known shoe and a highly detailed test exemplar have been the best form of evidence utilized in a comparison. This project relied heavily on the ability to properly document the outsole and obtain detailed test exemplars. The methods utilized captured the randomly acquired characteristics of the outsoles extremely well compared to the scanned outsole. However, it was surprising to find that the observed number of randomly acquired characteristics in each outsole was comparable.

In conclusion, further research should be performed in order to aid with the investigation of footwear and possibly tire tread examinations related to crime scenes.

#### Patterned Evidence, Footwear Evidence, Test Exemplars



### B24 Magneto-Archimedes Levitation (MagLev) Separation of Drugs

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**Learning Overview:** This presentation will inform the attendees on the process of MagLev and the application to separation of drugs of abuse. Attendees will identify the benefits associated with the use of MagLev to recover analytes sufficient for spectrophotometric analyses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community as this novel technique avails analysis of low-concentration drugs, in particular fentanyl and fentanyl-related substances.

Currently, forensic drug chemists face a challenge in identifying controlled substances in complex mixtures containing multiple drugs, adulterants, diluents, and other drug-related substances. Many forensic drug labs have formed a great deal of reliance upon chromatographic separations and identification, such as Gas Chromatography/Mass Spectrometry (GC/MS), which has relegated spectrophotometric techniques, such as infrared or Raman, to a minor role in day-to-day analysis with certain classes of drugs. In light of the complex mixtures typically encountered, the rise in fentanyl and fentanyl-related substances, particularly at low concentrations, necessitates the availability of additional separation techniques. Higher potency of this class of drugs, at times, dictates lower concentration in samples recovered by law enforcement and submitted to forensic labs. This method would enable recovery suitable for spectrophotometric evaluation for low-concentration drugs.

MagLev affords separation of hydrophilic drugs and related substances with little effort. The MagLev device is composed of like-facing NdFeB magnets mounted in 3D-printed brackets that are positioned above and below a polycarbonate cuvette. With the use of a non-polar solvent mixture of tetrachloroethylene and hexanes providing a solution of a gadolinium complex, hydrochloride salts of drugs and other water-soluble-related substances remain suspended within the cuvette in a vertical column. In the time span of 5 minutes to 30 minutes, these substances levitate according to their densities, which affords separation into discreet crystal masses in a restorative process. These crystal masses were then recovered via Pasteur-pipette while still under magnetic forces using a specially designed access port through the top of the device chassis. These crystal masses are then transferred to laboratory filter paper and dried of residual paramagnetic solution with acetone and vacuum dried in preparation for spectrophotometric examination via infrared or Raman spectrophotometry. These crystal masses were then successfully characterized via Fourier Transform Infrared (FTIR). Experimental separations were performed on methamphetamine HCl, cocaine HCl, heroin HCl, fentanyl HCl, acetylfentanyl HCl, and benzylfentanyl HCl. In addition to these reference materials, the technique was applied to commonly encountered mixtures with adulterants and diluents. One such example is a mixture composed of 1.3% fentanyl HCl, 2.6% heroin HCl, and 96.1% lactose. Due to the differing densities of each substance, the crystal masses of each levitated to a different height presenting separation. These were then recovered using a Pasteur-pipette for spectrophotometric characterization. MagLev provides a resilient separation of hydrochloride salts of drugs and most respective adulterants and diluents based upon their densities while suspended in non-polar solvents. This technique is amenable for a great many commonly encountered drugs of abuse, requires no electricity, is low cost save for modest solvent purchases, and has a very small bench footprint.

#### Magneto-Archimedes, Drug, Separation



### B25 An Investigation Into the Use of Amino Acid Ratios to Distinguish Between the Hairs of Similar Individuals

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**Learning Overview:** After attending this presentation, attendees will gain an understanding of how amino acid concentration ratios of hair proteins can be used to distinguish between the hairs of demographically similar individuals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an alternate method of hair analysis for when DNA is not present in a hair sample and for when different hair samples appear similar under a microscope.

Hair is a very important piece of trace evidence to forensic scientists because it is commonly found at crime scenes and can be a vital piece of evidence in a criminal case. Currently, forensic hair analysis is performed by both Microscopic Hair Comparison (MHC) and DNA analysis, but they are not always reliable. For example, MHC is very subjective and is not supported with statistical analysis. On the other hand, DNA analysis can be problematic if there is not enough undamaged nuclear DNA to sample from or if the nucleated cells within a hair follicular tag are not intact or are not present. Therefore, a new technique of analyzing hair, such as proteomic analysis, must be developed to replace MHC and DNA analysis for instances in which no beneficial results can be obtained from those methods. In this study, amino acid analysis was investigated as a new method to analyze hair proteins.<sup>1,2</sup> Human hair is made up of several proteins, such as keratin, and these proteins can have slight genetic variations in the form of Genetically Variant Peptides (GVPs). These GVPs contribute to an individual's genetic variation because they can alter amino acid sequences.<sup>3</sup> This means that the amount of amino acids can vary in people's hair based on their genetics.

First, hair samples were washed with deionized water and methanol. Then, the hair proteins were broken down into their corresponding amino acids via acid hydrolysis with hydrochloric acid. After digestion of the hair proteins, norvaline, an amino acid not synthesized by humans, was added to the hair samples as an internal standard. The amino acids were then derivatized with N,O-Bis(trimethylsilyl)trifluoroacetamide and identified using gas chromatography/mass spectrometry. The amino acids present in hair were quantified relative to norvaline, and the values were used to construct amino acid ratios. The amino acid ratios of various hair samples from individuals of common demographics and from monozygotic twins are compared to distinguish one hair sample from many others.

Hair samples were obtained on various days and from various areas of the head. The aim was to show that people's amino acid ratios do not change based on when the hair samples were taken or where the hair samples were taken from the head. Initial results suggest that amino acid ratios of hair proteins do not change over a short period of time and do not change among different areas of the head. Next, hair samples were taken from two people who have similar demographics. The goal was to use amino acid ratios to distinguish between the persons' hairs. Preliminary data supports the hypothesis that hair amino acid analysis can be used to distinguish between demographically similar individuals. For example, one person's proline/valine ratio was three times higher ( $5.46 \pm 1.68$ ) than the same ratio in the other person's hair ( $1.84 \pm 0.27$ ). Differences were also observed for the phenylalanine/leucine ratio, with one person's ratio being seven times higher ( $0.51 \pm 0.07$ ) than the other person's ratio ( $0.07 \pm 0.02$ ). Other ratios that were different between the hairs include aspartic acid/threonine, serine/leucine, tyrosine/valine, and several others. However, a larger population must be investigated further, including multiple individuals with similar demographics as these two people, as well as comparing the hair amino acid profiles of monozygotic twins.

In conclusion, hair amino acid analysis has the potential to enhance the current identification methods of individuals. Although it is not an individualizing technique, it could help provide law enforcement with a person's demographics based on hair found at a crime scene. This could help to narrow a suspect pool or rule out specific individuals.

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1. Sirena Lam. *Investigating the Use of Amino Acid Ratios in Human Hair to Assist in the Differentiation of Individuals Using Gas Chromatography/Mass Spectrometry (GC/MS)*. (Thesis, University of New Haven, 2018).
2. Ayat H.B. Rashaid, Peter B. Harrington, and Glen P. Jackson. Amino Acid Composition of Human Scalp Hair as a Biometric Classifier and Investigative Lead. *Analytical Methods* 7 (2015): 1707-1718.
3. Glendon J. Parker et al. Demonstration of Protein-Based Human Identification Using the Hair Shaft Proteome. *PLoS ONE* 11, no. 9 (2016): 1-26.

#### Amino Acids, Hair Proteins, GC/MS





### B26 A Comparison of Area of Origin Determination of Impact Spatter From Vertical and Horizontal Surfaces

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**Learning Overview:** After attending this presentation, attendees will understand the value of using spatter stains on both horizontal and vertical surface stains when determining the area of origin from an impact event.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by expanding the amount of evidence that can be utilized in the field, as well as defining limitations of the method when applied to stains on a horizontal surface.

Traditionally, determining the area of origin of an impact event has utilized stains deposited on vertical surfaces. Recent research has determined that impact stains found on a horizontal surface in front of an event may be useful in the determination of area of origin, but its use is more limited.<sup>1</sup> To date, there has been no direct comparison of the calculations of the area of origin made from stains on both the vertical and horizontal surfaces surrounding the same event.

This experiment compares the area of origin determination from stains on vertical and horizontal targets, from the same event, to determine whether including measurements calculated from both surfaces provides similar results. To produce a consistent impact pattern, a ring stand was attached to a leveled horizontal surface and a five-pound weight was used to strike a 1.2 milliliters (mL) pool of defibrinated sheep's blood from a height of six inches. "Walls" were constructed from uncoated butcher paper and placed ten inches from the center of the blood pool. A butcher paper "floor" extended approximately ten feet in front of the blood pool. Impact events were conducted at four heights representative of four body positions: standing (5 foot 8.8 inches), sitting (3 feet 6 and a half inches), kneeling (1 foot 6 and a half inches), and lying prone (6 and a half inches). A total of 120 patterns were created with ten events per height. Individual stains on each wall and floor pattern were selected to be measured. Only well-defined ellipses that indicated the appropriate directionality from the impact site were selected. Based on previous research, elliptical stains that were only within one meter of the impact site on the horizontal surface were used for area of convergence and area of origin determination.<sup>1</sup> The length and width of each was measured using a digital microscope. A Leica® Bosch GLM 35 laser measuring device was used to measure from the leading edge of each stain to the chosen area of convergence. The area of origin was calculated using a trigonometric formula. Results from a previous study, as well as this experiment suggest that current methods of impact determination used on vertical surfaces can be applied to horizontal surfaces within limitations.<sup>1</sup> The area of origin calculated from horizontal surfaces is similar when subjects that are in a lying prone position or kneeling on all fours to that calculated from vertical surfaces. However, as height increases (sitting or standing), finding suitable stains becomes increasingly more difficult and results are less accurate. Thus, calculating the area of origin from both horizontal and vertical surfaces may be useful in some cases, but caution should be used if a sufficient number of suitable bloodstains are not present.

#### Reference(s):

- <sup>1</sup> Rindom, Kristin Jenifer. *Measurement Determination of Blood Spatter on a Horizontal Surface*. Master's thesis, Emporia State University, 2019. Unpublished.

#### BPA, Horizontal, Vertical



### B27 An Analysis of Organic and Inorganic Gunshot Residues (OGSR and IGSR) Via Electrochemical Methods With Screen-Printed Carbon Electrodes and Nanoparticle Modifications

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**Learning Overview:** After attending this presentation, attendees will be able to understand the use of alternative analytical tools to detect Gunshot Residues (GSR) and to explain the advantages associated with electrochemical methods to improve detection capability, uncertainty, and speed of analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through the implementation of a rapid and comprehensive voltammetric GSR method for the dual detection of IGSR and OGSR. Practitioners will find a simple yet powerful technique that could offer timely investigative leads and speed up forensic investigations.

Following the discharge of a firearm, the compounds present in the propellant and primer, as well as residues from the cartridge and bullet, are expelled from the firearm under high pressure and heat. These compounds, globally termed GSRs, are then deposited in the vicinity of the shooting, including the hands and clothing of individuals. The detection of GSR can be a critical piece of evidence during a shooting incident and can provide valuable leads to investigators. However, the current analysis methods either lack sensitivity (as in color testing), take hours per sample (as in using Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS)), or are limited to detection of only the inorganic or the organic markers.

Electrochemistry improves upon these weaknesses by offering the simultaneous analysis of both IGSR and OGSR signatures, reducing run times to a few minutes per sample, offering qualitative and quantitative information, and providing lab-based and portable field testing. In this study, the analysis of OGSR and IGSR was performed using both Square-Wave Voltammetry (SWV) and differential pulse voltammetry in acetate buffer pH 4.5. A pre-concentration potential was applied before the analysis of the sample to improve the signal. The electroanalytical method was performed first using disposable carbon electrodes for the analysis of inorganics (lead, antimony, and copper) and organics (2,4-Dinitrotoluene [DNT], Diphenylamine [DPA], Nitroglycerin [NG], Methyl Centralite [MC], and Ethyl Centralite [EC]). The use of gold nanomaterials for the modification of electrode surfaces was investigated for the deposition of gold (Au) onto the working electrode (Screen-Printed Carbon Electrodes [SPCE]/Au). The use of bismuth films was also explored. Comparison of the performance of bare carbon, bismuth film, and gold surface working electrodes is presented in this study.

The following limits of detection were determined through statistical analysis of calibration curves as three times the standard deviation in the y-intercept divided by the mean in  $\mu\text{g/mL}$ : 0.09 (lead), 0.25 (antimony), 0.04 (copper), 1.28 (DNT), 1.17 (DPA), 0.52 (NG), 0.68 (EC), and 5.34 (MC). Analysis of GSR via gold deposition onto the SPCE working electrode resulted in improved limits of detection (in  $\mu\text{g/mL}$ ) of 0.012 (lead), 0.011 (antimony), 0.013 (NG), and 0.007 (DNT). The limit of quantitation for the organic compounds using the SPCE/Au was below  $0.050\mu\text{g/mL}$  with the relative standard deviation for repeatability and reproducibility below 2%.

A validation set of 180 samples collected from the hands of shooters ( $n=80$ , diverse ammunition and firearm) and non-shooters ( $n=100$ ) were run to assess the performance of the method, with overall accuracy better than 84%. Accuracy was estimated based on the number of true positives, true negatives, false positives, and false negatives.

This work demonstrated the ability of simultaneous detection of both OGSR and IGSR using electrochemical methods. Excellent improvements in sensitivity were shown for OGSR when utilizing a gold surface on the carbon working electrode. The sensitivity for OGSR is highly desirable due to the higher informative value of these compounds in associating a suspect to a shooting incident.

#### Gunshot Residue, Electrochemistry, Screen-Printed Carbon Electrodes



### **B28 The Utilization of Classical Forensic Methods in the Study and Preservation of Human History and Cultures, the Conservation and Restoration of Objects of Art and Artifacts, as Well as the Detection of Art Fraud and Counterfeiting**

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**Learning Overview:** The goal of this presentation is to further advance the utilization of well-established forensic science analytical methods and techniques in the study of historic preservation and art conservation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by assisting attendees to acquire the knowledge, skills, and ability to apply classical forensic science methods and techniques to the disciplines of historic preservation and art conservation.

Edmond Locard, a founding father of forensic trace evidence analysis, noted in 1929 that whenever a person commits a crime, there is always a mutual transfer of trace evidential materials between the people, places, and things involved in the crime. Locard established in his work, research, and writings that these traces can be effectively used to not only describe the people, places, and things involved in an event, but the incident itself. In other words, transferred trace evidence can be used to reconstruct the crime.

It turns out that the application of Dr. Locard's principle of mutual exchange, classical forensic science investigative rationale, well-established forensic scientific methodologies, and laboratory tools can be successfully applied in many other scientific endeavors. Relics such as fragments of textiles, minute pieces of stone, ceramic, pottery, glass, metal, and wood or any artifact of historical interest found at archeological studies of ancient sites can be documented, studied, analyzed, identified, and compared employing forensic methods and procedures.

Additionally, art-oriented scientific disciplines, such as historic preservation, scientific authentication, preservation and conservation of historically significant buildings, monuments, statuary, fine and decorative arts, can all benefit by applying the forensic approach to identity, individualization, and reconstruction.

Typically, preservation, conservation, and authentication projects start with an in-depth study of the subject's history and ownership of the artifact or object of art that is under study. In the case of a piece of fine art, provenance is often of prominent importance. Next, features, such as an artist's style and technique, are crucial to their endorsement. Other essential aspects, including the chemical analyses of the materials available during an artist's lifetime, are vital in determining the authenticity of a work. In many cases, less-scientific forms of proof, such as an artist's signature, the identification of a fingerprint, the advice of experts, or the opinion of a connoisseur, are the sole evidence of proof. These forms of evidence often lead to confusion and misidentification.

When questions remain concerning the methods and materials used to produce the work or the authorship of the work, the trace evidence embedded within the work remains a vital, untapped, valuable, gold mine of data and unbiased proof to acquire information concerning these artifacts, articles, and works and to determine the work's creation and creator.

This study presents real-life examples in which the Locardian principal, forensic investigative logic, and well-established forensic methodology can apply to the disciplines of historic preservation, fine and decorative art restoration and research studies, as well as conservation and restoration case studies. A number of studies of vital historical note, including the restoration of the original Statue of Liberty in 1983, the authentication of the missing 9/11 American flag, the identity of the American flag draped around the shoulders of the 1980 gold medal-winning USA Hockey team's goaltender, a research study of the dust in Napoleon's origin dress uniform, as well as several works of decorative and fine arts paintings, including the long-suspected last work of Jackson Pollock, are presented and fully elucidated.

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#### **Forensic Science, Historic Preservation, Art Conservation**



### **B29 The Chemical Composition of Gunshot Residue (GSR) of Commonly Encountered Samples in Shooting Events in Kosovo by an Optimized and Validated Scanning Electron Microscopy With Energy Dispersive X-Ray Spectroscopy (SEM/EDS) Method**

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**Learning Overview:** The goals of this presentation, based on the presented results and discussions, are to gain knowledge about the state-of-the-art in the field of GSR analysis and applied classification in different countries, to increase awareness of the forensic scientist regarding the chemical composition of ammunition encountered in shooting events in the Republic of Kosovo, to initiate GSR database development in Kosovo, and to initiate discussion on the classification of GSR particles currently applied in Kosovo.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exerting a positive effect by describing the current state of GSR analysis in the Republic of Kosovo and the capabilities of the legal science lab in the optimization and validation of the SEM/EDS method, as well as by critically summarizing the findings on the chemical composition of GSR samples commonly encountered in Kosovo. This initiated preliminary study on the GSR database in Kosovo could provide additional information to forensic scientists worldwide.

This work is focused on optimization and validation of the SEM/EDS method for inorganic GSR analysis in samples commonly encountered in shooting events in the Republic of Kosovo. The effect of accelerating voltage (12.5–30kV), working distance (8–14.2mm), spot size (3.5–8), acquisition time (1–30sec), and detector resolution (Amper time 0.12, resolution 179.88, Amper time 3.84, resolution 128.32) were optimized to obtain accurate and reliable GSR identification and composition data. The partially optimized method was validated according to the requirements of the American Society for Testing and Materials (ASTM) 1588-08. The repeatability and within lab reproducibility for Lead, Antimony, and Barium (PbSbBa) particles of 0.5 $\mu$ m diameter was 8% Relative Standard Deviation (RSD). The RSD of 100 total PbSbBa particles was 2% in a 4-week study by two operators. The trueness was estimated by bias and sensitivity. Mean sensitivity of the 4-week study was 95% (SD 2) and bias was -5% (SD 2), no false positive was detected, the number of false negatives was between 3 and 7, and the number of true positives was between 96 and 100. The partially modified ASTM 1588-08 method was proved fit for purpose.

Chemical composition of GSRs found in shooting events in Kosovo was determined by the validated SEM/EDS method. Both characteristic three component particles and consistent two components particles were considered. A survey on the chemical composition of particles from shooting events encountered in Kosovo in the past three years was presented.

The results have shown that recently non-toxic ammunition has been used in the Republic of Kosovo. The use of lead-free ammunition has spread worldwide; however, the classification of particles as GSR is still not widely agreed upon. On one hand, the ASTM standard applied in Kosovo states that PbSbBa has spherical morphology to classify particles as a GSR. On the other hand, the European Network of Forensic Science Institutes (ENFSI) standard applied in the European Union (EU) additionally included as characteristic for GSR the particles containing the phases of Gadolinium (Gd), Titanium (Ti), and zinc (Zn) or the phases of Gallium (Ga), Copper (Cu), and Tin (Sn). Currently, ASTM qualification is applied in Kosovo. However, careful study of samples from shooting events in Kosovo revealed the increasing number of cases in which lead-free ammunition was used. This fact requires reexamination of the standards in GSR forensic standards used in the Republic of Kosovo. Moreover, some non-routine elements were also detected in the studied samples. The comparison between GSR composition and chemical composition of cases or cartridges could provide additional information to forensic scientists.

#### **Gunshot Residues, SEM/EDS, Validation**



### B30 The Effect of pH on Presumptive Forensic Serological Testing

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**Learning Overview:** After attending this presentation, attendees will better understand how and if soil pH affects blood and semen stains. In addition, this presentation will investigate whether the length of time that semen or blood is exposed to soil affects presumptive test results.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing more detailed information on presumptive testing for blood and semen and if a negative result obtained after presumptively testing buried material could be the result of a false negative.

When clothing is found buried at a crime scene, a presumptive test will be conducted to determine whether biological evidence in the form of blood or semen stains is present. If the result is positive, evidence is sent for a confirmatory test, followed by DNA analysis. If the result is negative, in most instances, testing ceases. Presumptive serological testing is a cost- and time-effective way to determine if suspected biological fluid is present. However, presumptive testing is known to produce false positive results with some substances. What is less known is whether false negatives exist. This research attempts to answer the question: "If blood or semen was present on clothing exposed to soil, could the pH of the surrounding soil and the length of exposure cause a false negative result?"

Two hundred fifty 2cm<sup>2</sup> fabric swatches were prepared: 108 swatches were stained with 50μL of human blood and 108 swatches with 100μL of human semen. The presumptive tests chosen for this project were Acid Phosphatase for seminal fluid and Kastle-Meyer test for blood. To determine if soil pH would affect presumptive testing, acidic, neutral, and alkaline soil was prepared. Approximately 145g of each soil type was placed in its own plastic container, resulting in 66 containers of acidic soil, 66 containers of neutral soil, and 66 containers of alkaline soil. After the stains had dried in ambient conditions, each fabric swatch was placed into its own container and surrounded with soil. Positive controls (stained fabric left unburied) and negative controls (fabric buried but not stained) were also prepared. Soil containers were stored in the lab in darkness at 70°F. Fabric was left exposed to soil over the following intervals: 3 days, 7 days, 11 days, 30 days, 45 days, and 60 days. Five replicates of each fluid sample at each exposure time in each soil type was prepared, as well as a negative and positive control. Once a sample reached its designated exposure time, the fabric was removed from the container and any loose soil was cleaned off. Presumptive testing was conducted according to manufacturer's guidelines. Presumptive testing was also conducted on a positive and negative control.

Results show that soil pH does, indeed, impact serological screening results as does the duration of exposure. Blood stains in the neutral soil appeared more deteriorated when compared to stains from other soils, even when exposure time was greater. The reaction time and intensity of the results for both the Kastle-Meyer test and Acid Phosphatase test for neutral soil samples changed after the 11-day exposure period. The intensity of the result of the Kastle-Meyer test directly compared to the intensity of the blood stain. The Acid Phosphatase test result was in the last acceptable time range for color change (i.e., 60 seconds). Forensic serologists should be aware that buried evidence can impact screening tests. It is important for analysts interpreting screening test results on buried evidence to exercise caution when determining whether or not a weak result is a true negative.

#### Presumptive Testing, Serology, Soil pH



### B31 Analyzing Gamma-Hydroxybutyrate (GHB) by Fourier Transform Infrared Spectroscopy (FTIR) With Minimal Sample Preparation

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**Learning Overview:** After attending this presentation, attendees will understand how to detect the Schedule 1 controlled substance GHB and distinguish it from the non-scheduled Gamma-Butyrolactone (GBL) using FTIR.

**Impact on the Forensic Science Community:** Due to different legal classifications of GHB and GBL, it is imperative that forensic chemists have the tools to distinguish between the two substances. This presentation will impact the forensic science community by demonstrating one such tool, FTIR, to allow for such a distinction.

Typically, forensic drug laboratories often rely on Gas Chromatography/Mass Spectrometry (GC/MS) instrumentation for the separation and detection of controlled substances. However, GHB converts within the high-temperature injection port to the non-scheduled GBL. Due to different legal classifications of these substances, it is imperative that forensic chemists have the tools to distinguishing between the two substances.

One commonly used traditional method of analysis includes derivatization of GHB followed by GC/MS, stabilizing GHB from dehydration cyclization to GBL.<sup>1-3</sup> Derivatization is a somewhat lengthy process and requires the use of a toxic chemical—N,O-Bistrifluoroacetamide (BSTFA). Alternatively, GHB analysis can be performed by liquid-liquid extraction, requiring a firm grasp of solubility of GHB in sample matrix versus extraction liquid.<sup>4</sup> Unfortunately, this approach is complicated by the limited solubility of GHB and often yields too little recovered product to perform a confirmation. Finally, both <sup>1</sup>H and <sup>13</sup>C Nuclear Magnetic Resonance (NMR) have been reported for the identification of GHB in forensic analysis; however, many forensic laboratories do not have access to this instrumentation.<sup>5</sup>

The DC Department of Forensic Sciences Forensic Chemistry Unit currently uses the BSTFA derivatizing agent to confirm detection of GHB by GC/MS. Therefore, it was important to investigate if another method could be implemented to support routine casework, or at least provide another orthogonal method for confirmation. This research project focused on demonstrating the FTIR method using samples in pure liquid matrices. A consequence of this research was the demonstration of a more rapid and safer preparation method, as well as an efficient detection method for GHB and GBL using FTIR.

A validation of the sample preparation and analytical detection was performed as part of this research project. The validation was based off a previously validated cocaine method (for base vs. salt form) in determining selectivity, matrix effects, precision, limit of detection, and robustness for GHB and GBL. The analysis proved successful in this work, except for complications found in some complex matrices. The different solvents used to study matrix effects were: (1) ethanol, (2) methanol, (3) water, and (4) glycerin. It was discovered that when GHB is mixed in these matrices, the broad hydroxyl peak around 3,300 cm<sup>-1</sup> provided a challenge in resolution of characteristic functional groups within the spectrum. This effect can be addressed; however, by systematically removing the matrix via nitrogen evaporation. This process was demonstrated with water in the present study, to be expanded upon in future studies to the other matrices. Further, future research will focus on addressing sugar-saturated solutions, as GHB is often knowingly or unknowingly consumed in sugary drinks, such as juice, mixed alcoholic drinks, or sodas.

#### Reference(s):

1. Gamma-Hydroxybutyrate/ Butyric Acid. *SWGDRUG (Monograph, May 2005)* <http://www.swgdrug.org/monographs.htm#G>.
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3. Ciolino L.A., Mesmer M.Z., Satzger R.D., Machal A.C., McCauley H.A., Mohrhaus A.S. The chemical interconversion of GHB and GBL: Forensic issues and implications. *Journal of Forensic Sciences* 46, no. 6 (2001): 1315-1323.
4. Chappell J.S., Meyn A.W., Ngim K.K. The extraction and infrared identification of gamma-hydroxybutyric acid (GHB) from aqueous solutions. *Journal of Forensic Sciences* 49, no. 1 (Jan 2004).
5. DeFrancesco J.V., Witkowski M.R., Ciolino L.A. GHB free acid: I. Solution formation studies and spectroscopic characterization by <sup>1</sup>HNMR and FT-IR. *Journal of Forensic Sciences* 51, no. 2 (Mar 2006): 321-329, <https://doi:10.1111/j.1556-4029.2006.00073>.

#### Gamma-Hydroxybutyrate (GHB), Gamma-Butyrolactone (GBL), Infrared Spectroscopy (FTIR)



### B32 Presumptive Test Compatibility With Efficient DNA Collection Swabs

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**Learning Overview:** After attending this presentation, attendees will be better informed regarding the fact that the collection of stains at crime scenes and preservation of the integrity of the collected biology evidence after collection are critical components impacting the success of downstream chemical and molecular biology assays. While an important part of the evaluation of biological evidence, presumptive tests and whether there are any inhibitory effects associated with the swabs used to collect evidence have rarely been validated. Over the past few years, there have been advances in the collection tools (i.e., swabs; notably the advent of nylon swabs that presumably should be inert regarding downstream assays). However, the glues, process residuals during manufacturing, and any additives (especially those intended to preserve DNA) may impact performance of chemical or enzymatic presumptive tests. Therefore, swabs should be evaluated for presumptive tests compatibility, just as the rest of the tests in the laboratory workflow.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an understanding that all aspects of forensic biological analyses require validation. In particular, the development of superior performance synthetic swabs requires consideration that the additives or manufacturing components may impact presumptive test results. Therefore, these swabs, if used for sample collection, should be validated for the intended use.

Swabs should be tested to determine if there are any effects from the swabs on downstream presumptive tests. Based on the demonstrated success of DNA collection and DNA yield in the studies by Comte et al. and Sherier et al., the COPAN® Italia 4N6FLOQSwabs™ were selected to develop an effective test system.<sup>1,2</sup> These swabs are similar except that some versions contain an anti-microbial reagent. Thus, a controlled experiment between swab types can be performed for this initial test design. If the process is effective, then it could be used for more comprehensive testing among a variety of swabs, if needed. Blood, semen, and saliva stains on glass slides were prepared. The concentrations were neat and diluted (up to 1:99). The stains were allowed to dry overnight (minimum) before testing. Standard presumptive tests for each stain type (blood: leuco malachite green, saliva: RSID saliva; semen: acid phosphatase) were employed. These three presumptive tests were selected because they span the range of chemical (peroxidase-like catalytic activity of hemoglobin), enzymatic activity (acid phosphatase), and antibody-based (amylase detection) assays. The results were compiled as positive/negative and with an arbitrarily designed 1-4 scale. Preliminary testing supports that 4N6FLOQSwabs™ are inert with regard to these presumptive tests.

#### Reference(s):

1. Comte, J. et al. (2019) Touch DNA collection—Performance of four different swabs. *FSI Genetics* (in press).
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#### Presumptive Tests, 4N6FLOQSwabs™, Validation



### **B33 A Comparison of DNA Typing Success in Compromised Blood and Touch Samples Based on Sampling Swab Composition**

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**Learning Overview:** After attending this presentation, attendees will understand the impact of time and temperature on DNA analysis of samples using both cotton and a proprietary swab designed to minimize degradation of forensic samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an opportunity to evaluate a novel sampling product for forensic DNA evidence and promote more research about and awareness of preventing DNA degradation at sampling and during storage.

Sample collection at the crime scene can introduce variations in DNA recovery, both based upon the substrate or material used as well as the storage conditions after collection. Both the substrate and the body fluid deposited on the evidence can harbor microbial populations, which can be co-collected onto the swab and degrade the sample even during drying and storage, and before DNA extraction can be performed. The purpose of this study was to evaluate and compare standard cotton swab collection with the Bode BioSafe™ swab, which includes both a desiccant at the swab head as well as proprietary bacteriostatic compounds included to prevent degradation of the sample.

Venous blood samples were collected from five donors into sodium heparin tubes, and contact samples from ten donors were deposited onto both porous (cotton fabric for both blood and touch) and non-porous (linoleum flooring and computer keyboards, respectively) surfaces and allowed to dry. The samples were then swabbed with a standard cotton swab or the BioSafe™ swab and stored at room temperature, 37°C or 55°C for 30, 90, or 180 days. After the elapsed time, DNA was isolated and quantified from all samples, and DNA profiles developed from select samples.

DNA yields varied from 80–190ng and 10–200ng for blood samples deposited on linoleum and cotton, respectively, regardless of swab method used. While no trends were observed in comparing the DNA yields of samples extracted from blood collected with either swab type, degradation indices tended to be lower for BioSafe™ as compared to cotton swabs. Contact DNA samples yielded between 0.1–1.5ng and 0.5–5ng on T-shirt and keyboard samples, respectively, with higher yields found with samples swabbed using the BioSafe™ swabs on both substrates, and lower indications of degradation with the BioSafe™ swabs. Because touch or contact samples are known to harbor higher bacterial loads than blood in addition to the free DNase enzymes known to inhabit keratinized epithelium, the bacteriostatic, desiccating, and enzyme-inhibiting capability of the BioSafe™ swabs may prove to result in better DNA profile outcomes than the standard swabbing procedure.

#### **Swab, Touch, LCN DNA**





### **B34 A Comparison of DNA Profiles Recovered From Cotton Versus Nylon-Flocked Swabs From Postcoital Cervicovaginal Samples**

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**Learning Overview:** The goal of this presentation is to illustrate the differences observed in DNA recovery efficiencies between traditionally used cotton-tipped swabs and newer nylon-flocked swabs from postcoital cervicovaginal samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information that could potentially improve the standard practices and tools used as part of a sexual assault kit.

Cotton-tipped swabs are routinely used as a method of collecting biological material by medical personnel and forensic investigators. Standard practices include collecting vaginal swab samples from a female victim as part of a sexual assault kit. Swabs used to collect biological material should be able to maximize the cellular material collected, then elute intact cells for DNA extraction and profiling. A swab with a higher extraction and recovery efficiency can lead to more DNA available for genotyping. The differences in swab morphology between cotton-tipped swabs (considered wound swabs) and the hydrophilic open fiber structure of the flocked swabs may account for the higher rates of sample elution from flocked swabs.<sup>1</sup> This study aims to compare male profiles obtained from vaginal samples taken with cotton-tipped swabs and nylon-flocked swabs.

Following Institutional Review Board (IRB) approval, sets of four vaginal swabs were collected from sexually active, heterosexual participant couples. The couples varied in age, menstrual cycle, and birth control methods. Swabs were collected over three distinct postcoital time intervals, with the cotton and the nylon-flocked swabs being inserted concurrently following the National Institutes of Justice (NIJ) recommendations for sample collection.<sup>2</sup> Control swabs of the vaginal cavity were taken after a sexual abstinence period of at least seven days and collected immediately prior to intercourse. Vaginal swabs were collected after 72, 120, and 168 hours postcoitus with ejaculation. Each collection was based on a separate act of intercourse. Cheek swabs of the male and female participants were collected to obtain reference profiles. The reference swabs were extracted using a 5% Chelex<sup>®</sup> method. The vaginal swabs were extracted using QIAamp<sup>®</sup> DNA Investigator Kit. Both reference profiles and vaginal swab extractions were amplified using the PowerPlex<sup>®</sup> Y23 System. For one 72-hour sampling period, the profile obtained from the cotton swab showed 3 of 22 (13.6%) alleles, while the profile obtained from the nylon swab showed 12 of 22 (54.5%) alleles. This same couple also completed the 120-hour sampling period, with the cotton profile returning 2 of 22 (0.9%) alleles and the nylon profile having 13 of 22 (59%) alleles present. For a second 72-hour sampling period by different participants, the cotton profile returned 16 of 22 (72.7%) alleles, while the nylon profile had all 22 alleles present. The 72-hour sampling period by a third couple returned full profiles (22 of 22 alleles, 100%) for both the cotton and the nylon swabs.

These results provide data that could be used to improve the collection methods used in a sexual assault kit. The improvement of the swabs used in postcoital vaginal sampling could have positive downstream implications when attempting to obtain a DNA profile.

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- <sup>1</sup> Benschop C., Wiebosch D., Kloosterman A., Sijen T. Post-coital vaginal sampling with nylon flocked swabs improves DNA typing. *J Forensic Sci Int:Gen* 2010;4:115-121.
- <sup>2</sup> National Institute of Justice. *National best practices for sexual assault kits: A multidisciplinary approach*. 2017. NIJ 250384;15-19.

#### **Sexual Assault Collection, Nylon-Flocked Swabs, Cotton Swabs**



### **B35 Direct Polymerase Chain Reaction (PCR) Using MicroFLOQ® Direct Swabs With a Modified QIAGEN® Investigator 24plex GO! Protocol From Decomposing Human Remains for Disaster Victim Identification (DVI) Applications**

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**Learning Overview:** After attending this presentation, attendees will understand the performance of a direct PCR workflow for DVI samples using the microFLOQ® direct swabs in conjunction with the QIAGEN® Investigator 24plex GO! kit. This project compared a direct and traditional workflow for processing decomposing human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an alternate workflow for processing DVI samples. DVI relies on rapid identification of decomposing human remains, often in remote areas without access to storage facilities. Collection of biological material using swabs may prove easier, more efficient, and more amenable to storage in harsh conditions. MicroFLOQ® direct swabs have been identified as a potential alternative for more rapid collection and processing of DNA in forensic and DVI situations.

4N6FLOQSwabs™ Genetics and microFLOQ® direct swabs were used to collect DNA from red muscle via an incision in the arm or leg of a decomposing human cadaver. Traditional DNA processing with the Genetics swabs was compared to a direct amplification strategy using the microFLOQ® swab coupled with the Investigator 24plex QS GO! Kit. Additionally, both swab types were evaluated for their ability to store DNA for up to three months. The direct amplification strategy was optimized by pre-treating the swab (washing, vortexing, lysis) prior to amplification and slightly modifying the cycling parameters. As an alternate method, the microFLOQ® swabs were used to sub-sample DNA stored on the Genetics swabs.

Results indicate that both swab types were able to store DNA at room temperature. Short Tandem Repeat (STR) success rates of traditional and direct PCR methods were comparable but were highly dependent on the stage of decomposition and the sample location. Up to day ten, full profiles were obtained using both processing methods with samples taken from the leg for up to three months of storage at room temperature. Full profiles were obtained from day 13 and day 20 using traditional methods, while partial profiles were obtained on day 13 using microFLOQ® swabs and subsampling. The Quality Sensor (QS) markers were used to assess sample quality. Interestingly, the QS markers indicated that even after pre-washing the microFLOQ® swabs, there was still inhibition present in the amplification for microFLOQ® swabs that were dried overnight. However, when the microFLOQ™ swabs were processed within hours of swabbing or used to sub-sample, there was less-to-no inhibition indicated by the QS markers and profile completeness improved.

Overall, microFLOQ® swabs in conjunction with the GO! Kit facilitated direct processing in the laboratory from decomposing remains.

**Disaster Victim Identification, Direct Amplification, microFLOQ®**



### B36 Criminalists at the Crime Scene: Where Do They Come From and Where Can They Go?

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**Learning Overview:** After attending this presentation, attendees will better understand the myriad ways organizations recruit crime scene investigators, with emphasis on the variation in required education, training, and experience. The goal of this presentation is to encourage attendees to address what is needed to be a successful crime scene investigator and what educational and career paths are necessary to ensure a sustainable solution to crime scene staffing.

**Impact on the Forensic Science Community:** Crime scene investigation and reconstruction are scientific endeavors requiring individuals educated and trained in the principles of criminalistics, as well as the fundamentals of critical thinking and reasoning. This presentation will impact the forensic science community by explaining that the technical “bag and tag” approach is not sustainable for the complexity of today’s cases and the increased momentum toward robustness and accountability in forensic science endeavors. Panelists from the United States, Europe, and Australia will share their experiences with these different solutions, and provide opinions on what the critical elements for success are and what still needs to be improved on a system scale in order to ensure that forensic science is used to its full potential at the crime scene.

Attendees will be encouraged to provide their own experiences and concerns in an effort to facilitate discussion and broaden the scope of the presentation topic.

Crime scene work is arguably the most critical part of the forensic science process. The changing crime and security environment, including increased traceability (e.g., more varied trace sources, larger volume of traces, pace, blurring of physical and digital lives, etc.), continue to add complexity to crime scene work. Developments in quality assurance and accreditation within the discipline of crime scene investigation have increased the pressure on these actors to reflect on how to validate their standard operating procedures and how to demonstrate the competence of their staff.

Some jurisdictions follow a testing lab model based on the “bag and tag” approach (i.e., the scene investigator collect traces, perhaps triaging the packaged items, and then sends “test tubes” to a forensic laboratory that is increasingly disconnected from the forensic science problems). In this model, crime scene investigation is often undertaken by sworn officers and the scientific education, training, and experience of these individuals can be quite diverse.

In some jurisdictions, there has been a push for civilian scientists at the crime scene, with science graduates being recruited. This alone does not necessarily solve the problem—a science graduate does not necessarily translate to an effective examiner. Only a few of these individuals manage to find a path of appropriate career progression, perhaps because the culture remains that to progress you must be a sworn member of law enforcement.

In other jurisdictions, generally those with an integrated lab/forensic service within police agencies, the situation has been more seamless, and there is less obvious distinction between sworn and unsworn members of the crime scene investigation unit. An example of this is the Australian Federal Police (AFP) who now apply a problem-oriented/consultancy model within their organization. Crime scene investigation is undertaken by sworn and unsworn employees, and there are ample opportunities for people to move from one space to the other.

The critical point is to have staff with the appropriate education, skills, and abilities to do the job properly. If the crime scene poses a scientific problem, then appropriately trained scientists are necessary to provide the solutions.

The feature of sworn vs. unsworn crime scene staff may seem to be of importance mainly for law enforcement organizations, but it can also have wider implications on the staffing and oversight of crime scene units under diverse management models. How can law enforcement attract, screen for, and retain critically thinking scientists for crime scene work? Is the strategy to recruit scientists and educate/train them to operate in the context of police work or to recruit police officers and educate/train them to be scientists? How can these strategies be successful? What positions can sworn or unsworn criminalists apply for when they want to or need to move on from operational crime scene work? What role can and should the forensic laboratory play in this framework?

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#### Crime Scene, Competence, Career Path



### B37 The Utilization of Sex Hormone Antibodies for Screening and Separation of Trace Biological Mixtures

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**Learning Overview:** After attending this presentation, attendees will understand: (1) how to optimize staining of epithelial skin cells with antibodies; (2) how to assess binding efficiency of antibody probes; and (3) if pairing antibodies to different molecular targets enhances the signal intensity or facilitates more distinct staining patterns for male versus female epithelial cells.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the novel application of fluorescently labeled antibodies targeting testosterone, Dihydrotestosterone (DHT), and estradiol molecules to selectively stain epithelial skin cells in trace biological mixtures. Using these antibodies to screen for male epithelial cells is an innovative concept that will benefit forensic biology units tackling touch/trace mixtures. Screening for the presence of male cells and separating them from female cells could eventually be applied to casework samples in order to simplify complex touch/trace mixtures or eliminate these mixtures altogether prior to forensic DNA analysis.

In recent years with the increased sensitivity of DNA analysis instrumentation and Short Tandem Repeat (STR) typing kits, evidence submitted to forensic laboratories for DNA analysis has shifted such that a large proportion consists of “touch” or trace evidence. Moreover, touch or trace DNA mixtures constitute one of the most difficult types of evidence to successfully profile and interpret. If male cells can be differentiated by antibody staining from female cells, this could be exploited to separate out contributors in a touch or trace mixture and increase the success rate of profile interpretation. Testosterone, the primary male sex hormone responsible for producing male phenotypes, is a potential antibody target due to its abundance in males at approximately ten times that of females in blood serum levels and its long-term stability in biological samples.

In this study, skin epithelial cells were used to model touch or trace mixture evidence, which constitutes half of the casework evidence items submitted for DNA analysis. For the optimization of antibody staining, male and female skin epithelial samples from the same donors were incubated and hybridized with fluorescently labeled anti-testosterone, anti-DHT, and anti-estradiol antibody probes separately, each at varying concentrations. Antibody binding efficiency was assessed by analyzing stained single-source male, female, and control epithelial skin cells through flow cytometry to determine if the staining was specific to either cell population compared to the unstained control. The objective was to maximize probe binding to male cell populations and the selective labeling of male and female cells. Once an optimal staining condition was established, it was tested across several individuals from each sex. If an improved signal was observed, as demonstrated by an increased median fluorescence and separation between male and female samples, then the testing moved forward to Fluorescence Activated Cell Sorting (FACS) analysis.

The results for testosterone labeling showed an increased median fluorescence for male cell populations compared to female. Staining with anti-DHT probes resulted in only a slight shift in median fluorescence from unstained to stained cells and showed no separation in median fluorescence between male and female cell populations. The results for estradiol antibody probes showed an increased median fluorescence separation between male and female cell populations. When testosterone and DHT antibodies were used simultaneously, the median fluorescence increased between unstained and stained cell populations. However, there was not a large separation in median fluorescence between male and female cell populations. Overall, these results suggest that antibody probes targeting testosterone and estradiol may be used to differentially label contributor cell populations in touch or trace mixture samples. This can potentially be used to presumptively screen evidence samples for the presence of either cell population or as the basis for separating cell populations based on fluorescence prior to DNA profiling.

#### DNA Mixtures, Touch/Trace Evidence, Cell Separation



### B38 Classification of Body Fluid Source in Dried Samples Using a Panel of MicroRNAs (miRNAs)

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**Learning Overview:** After attending this presentation, attendees will understand how miRNAs can be of significant value for body fluid identification in forensic casework. Attendees will understand that specific miRNA markers can distinguish different body fluids, and that miRNAs may be a better molecular-based method for the identification of body fluids than the use of current serological tests, which are based on enzymatic activity and are often prone to false positives.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the evidence for miRNAs as molecular markers for body fluid identification continues to build strength.

MiRNAs are small non-coding RNAs, 18–25 nucleotides in length, that have been identified and evaluated as potential markers for the identification of forensically relevant body fluids. There is significant interest in the use of miRNAs for forensic casework because of their short length and high resistance to degradation. They have also been shown to co-extract and be detectable in DNA extracts, which could make the use of miRNAs a more streamlined and easily implementable molecular body fluid identification method than other described methods.

Candidate miRNAs were identified through high-throughput sequencing of the miRNome and quantitative Polymerase Chain Reaction (qPCR) panel analyses of differential expression patterns in venous and menstrual blood, vaginal secretions, saliva, feces, urine, perspiration, and semen. Candidate miRNAs were identified and subsequently further validated using population sample sets from each biological fluid, ultimately identifying seven miRNAs to identify blood, semen, menstrual secretions, saliva, feces, and urine. This panel of miRNAs includes a pair of endogenous reference markers that provide normalization of miRNA expression without evaluation of the RNA quality or known input quantity. miRNA expression is detected using Reverse-Transcription quantitative PCR (RT-qPCR) to identify and differentiate dried body fluids of a volume and type as may be collected from the scene of a crime.

Each of the seven miRNA markers in the miRNA panel was analyzed in 50 samples of blood, saliva, vaginal fluid, urine, and semen samples and, together with previous data, were used to construct a classification regression tree that provides likely sample classification based on its relative expression of each candidate. Identification of the biological fluids was found to be reliable across population samples of mixed ages, ethnicities, and gender, with 80–100% of the unknown samples classified correctly, depending on the body fluid in question.

In conclusion, the classification tree developed in this work demonstrated high accuracy in identifying the biological source of unknown samples. This complements previous research confirming consistent miRNA expression in compromised samples, and a low limit of detection of  $10^4$ – $10^5$  copies. Consequently, the miRNA panel can provide robust, accurate identification of six biological fluids, with demonstrated utility for implementation into forensic casework.

#### Body Fluid Identification, MiRNA, MicroRNA



### B39 A Determination of the Optimal Method for the Detection of Vaginal Fluid

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**Learning Overview:** The goal of this presentation is to demonstrate the methods that have proven to differentiate vaginal fluid from other body fluids, such as saliva and urine.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the foundation for a potential confirmatory test for vaginal fluid, and so provide context for sexual assault investigations.

With the advent of DNA and the potential to be corrupted by bias, many people, including forensic scientists, have forgotten the importance of applying context to the evidence of a particular case. DNA may be present at a crime scene, but without context, that DNA evidence may be interpreted incorrectly. Identifying the body fluid from which the DNA came can provide information of how the DNA came to be there. Forensic science literature presents a number of tests to confirm the presence of various bodily fluids; however, such a test for vaginal fluid is still needed.

A confirmatory test for vaginal fluid will provide critical context for forensic casework, especially in sexual assault cases where no semen is present. In this study, different methods were examined to detect and confirm the presence of vaginal fluid on “used” condoms donated by women of different ages and then tested over periods of time. Following Institutional Review Board (IRB) approval, multiple samples were obtained from more than 20 volunteers. After extensive validation, a tandem method is proposed that provided robust results over all demographic groups tested.

The first method utilizes the Periodic Acid-Schiff (PAS) reagent to stain glycogen present in vaginal epithelial cells in a histochemical reaction.<sup>1</sup> The reagent proved to be an improvement over Lugol’s iodine, which was more commonly used in forensic labs.<sup>2</sup> Of 20 vaginal fluid samples tested on women ranging in age from 24–82 years, all produced positive results with no false positives observed with corresponding saliva and urine dried stains. These visual results were bolstered by using cellSens software with an Olympus® Polarized Light Microscope to photograph the stained slides and measure the Red, Green, Blue (RGB) values of each photo.

The second method utilizes real-time Polymerase Chain Reaction (PCR) to amplify genetic sites in three bacteria typically associated with the vaginal cavity: *L. crispatus*, *L. iners*, and *L. gasseri*.<sup>3</sup> This method also proved effective; while some of the bacteria were also present in saliva and urine, more amplified product was observed in vaginal fluid samples, as well as used condom samples.

Not only do these results bring to light more effective detection methods for vaginal fluid, but they also provide the foundation for a potential confirmatory test that could be used in serology laboratories Utilizing and validating these methods is vital for aiding in sexual assault investigations. The detection of vaginal fluid in cases could provide the context needed to solve these heinous crimes.

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**Periodic Acid-Schiff, *L. crispatus*, *L. gasseri***



### **B40 Forensic Body Fluid Identification Using Microbiome Signature Attribution Through 18S Recombinant DNA (rDNA) High-Throughput Sequencing**

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**Learning Overview:** After attending this presentation, attendees will have a better understand of eukaryotic communities associated with forensically relevant human body fluids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by developing an improved method for the accurate identification of body fluids as an alternative to current serological techniques. While the human microbiome has been used for identification purposes in the past, high-throughput sequencing has allowed for a more detailed and rapid analysis of microbial communities.

The conception of the Human Microbiome Project advanced the understanding of bacterial communities in the human body, and previous research has established that unique microbial signatures can help distinguish each body fluid. While these signatures have been developed for the prokaryotic microbiome, the next step is the examination of the eukaryotic microbiome. Eukaryotic signatures could provide a greater specificity and statistical weight when discerning between body fluids. These microbial markers can be implemented to develop a confirmatory assay for body fluid identification that works in tandem with other DNA-based methods in the forensic workflow.

Using an approved Institutional Review Board (IRB) protocol, 100 samples each of urine, feces, saliva, vaginal fluid, menstrual blood, and semen were collected. The semen and urine samples were first collected in a container, then dried onto sterile cotton swabs at room temperature. Saliva, vaginal fluid, menstrual blood, and feces were collected onto cotton swabs and dried at room temperature. DNA was isolated and quantified using DNA extraction methods commonly used by the forensic community. The V9 region of the 18S rDNA was amplified using dual-index strategy as described by Kozich et al.<sup>1</sup> Amplified products were purified, quantified, and pooled in equimolar concentration for paired-end sequencing on the MiSeq® FGx sequencing platform. The sequences will be analyzed using mothur version 1.39.4, and R version 3.4.0.<sup>2,3</sup> Ensemble subspace classification methods will be developed for the identification of body fluid samples in a single test either alone or in combination with bacterial signatures associated with these body fluids.

The 18S rDNA amplification resulted in two amplification products for most of the body fluid samples. Only those fragments of the target size were gel extracted and purified. For the same samples, microbial signatures based on 16S rDNA sequencing showed that except for female intimate samples (menstrual secretions, vaginal secretions, and female urine), the bacterial structure was significantly different between different body fluids. Most probably, an ensemble subspace classification method developed based on combined bacterial and eukaryotic community structure data will be able to differentiate even female intimate samples.

In conclusion, this study will highlight eukaryotic community structure associated with a large number of human biological samples and will develop a method that will be able to identify all forensically relevant biological samples in a single test.

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#### **Microbiome, Eukaryotic, Body Fluid Identification**



### **B41 Integration of Microchip Electrophoresis and a Validated Messenger RNA (mRNA) Panel as a Novel Approach to Forensic Body Fluid Identification**

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**Learning Overview:** After attending this presentation, attendees will better understand a simple method for microchip electrophoretic separations, the use of a novel mRNA panel for differentiation body fluids, and how the integration of these two scientific advances applies directly to forensic body fluid identification. This information will present a novel alternative to current methods used in forensic laboratories and literature.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a simple, yet specific, alternative method for body fluid identification that can be easily integrated into the forensic workflow, is automated to minimize user intervention, and can produce genetic profiles from mixture samples that are comparable to those generated from commercial genetic analyzers.

This research aims to develop a microfluidic approach to forensic body fluid Identification (bfID) using an mRNA panel coupled with electrophoretic separation on a centrifugally driven microfluidic device. The Institute of Environmental Science and Research (ESR) has developed a method for detection and differentiation of body fluids in an unknown sample. The approach is based on amplifying mRNA targets specific to individual body fluids, then electrophoretically separating the amplicons. Traditionally, a commercial genetic analyzer is used for separation of fragments by capillary electrophoresis. However, electrophoretic separation can instead be carried out on a microfluidic device to decrease required assay time, cost, and user intervention relative to use of a commercial instrument.

Genetic material (mRNA) was extracted from various samples containing either a single fluid (blood, semen, saliva, vaginal fluid, or menstrual blood) or a mixture, amplified, then separated by size using either a commercial genetic analyzer (3500xl or 3130xl), or a novel microfluidic device was developed for separation of the mRNA targets. Sample injection time and channel temperature for the microfluidic approach were optimized for improved DNA fragment resolution with on-disc microfluidic electrophoresis. To determine the optimal sample injection time for an on-disc electrophoresis, different electrokinetic sample injection times prior to separation were evaluated to determine the minimal amount of time needed to produce acceptable peak heights (above 1,000 Relative Fluorescence Units [RFU]). This study also evaluated whether heating of the separation domain/polymer was necessary for sufficient peak resolution and separation, with the temperature adjusted via an embedded Peltier thermoelectric device. Analysis of the resulting electropherograms indicated that heating of the separation channel did not have a large impact on the resolution or separation of the peaks in the electropherograms. To determine detection sensitivity of the on-disc platform, two single-source samples for each body fluid were diluted to various extents (1:2, 1:6, 1:10, or 1:14) prior to injection and electrophoresis. This demonstrated an on-disc platform sensitivity that was comparable to current bfID techniques. The fluorescence signal from all peaks was detectable through 2X and 4X dilution for the majority of the body fluids, although, some drop-out was observed.

In conclusion, this study has provided evidence for an on-disc microfluidic separation of a novel mRNA body fluid panel as an alternative to current bfID techniques that demonstrates detection of individualized targets for all five body fluids, detection of all peaks for mixture fluid samples, and rapid separation times (~8 minutes). This on-disc separation provides genetic profiles that are comparable to “gold standard” genetic analyzers used in forensic laboratories, based on peak heights and ratios. In addition, the on-disc platform was able to consistently separate all peaks in the mixture samples and show detection of highly diluted amplified samples. The next steps will be to optimize amplification of the mRNA targets on-disc and integrate the amplification and separation domains to allow for full automation after lysis of a sample for a “sample in-answer out” device.

#### **Microchip Separation, Body Fluid Identification, Mixture Analysis**





### **B42 Advances in the Development of Portable Surface-Enhanced Raman Spectroscopy (SERS) for Rapid, Sensitive, Confirmatory Identification of Human Body Fluids**

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**Learning Overview:** After attending this presentation, attendees will have learned that SERS has the attributes to become a transformative technology that can robustly detect and identify trace amounts of human body fluids in minutes employing an easy-to-use single platform that offers unprecedented sensitivity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting how recent advances in the use of a SERS-based methodology for rapid confirmatory identification of dried blood, semen, saliva, vaginal fluid, menstrual blood, and urine from a single portable platform is ready to be employed by forensic scientists at crime scenes and in forensics labs. No such capabilities currently exist in the forensic science community.

Body fluids, such as blood, semen, vaginal fluid, and saliva are among the most important forensic evidence collected at crime scenes. An ideal detection and identification platform for these fluids is confirmatory, rapid, sensitive, highly specific, portable, and easy-to-use, providing crime scene investigators with maximal time for subsequent law enforcement activities. Furthermore, non-destructive and/or highly sensitive techniques are desirable for forensic identification because they leave sufficient amounts of trace biological evidence for other detection platforms, in particular for DNA testing. This presentation demonstrates how recent advances in the development of SERS-based procedures allows SERS to satisfy these forensic attributes in a portable single-platform instrument.

In a new sample preparation protocol, SERS spectra of dried blood, semen, saliva, vaginal fluid, urine, and menstrual blood have been readily obtained using a simple 50% acetic acid (HAc) extraction step. This results in larger signals, and better differentiation, than water or saline extraction. Perhaps most significantly, this novel methodology allows the blood identification signature to be dominated by the SERS spectrum of hemoglobin and minimizes variable background non-specific protein contributions. Identification of unknown body stains are accomplished by unique statistically based identification procedures resulting in analytical sensitivity and specificity >95% for all body fluid classification analyses when combined with this new sample preparation protocol.

High sensitivities have been routinely achieved with this SERS identification and detection procedure. For example, SERS spectra with excellent signal-to-noise of human blood diluted by  $10^4$  in water can be readily obtained. A dramatic and practical demonstration of SERS sensitivity is further illustrated by spreading a solution of 10 $\mu$ L of blood in 1mL of water over a 1 sq. ft. piece of glass, rubbing a swab with 50% HAc over a 1 sq. in. area, then touching the swab to the SERS chip, resulting in a strong SERS blood spectrum. By using a subtraction methodology, this study has been able to provide confirmatory identification of dried blood stains after they have been presumptively identified by luminol emission.

In another key advancement for practical and routine use of this technology, cotton swabs have been developed for the acquisition of SERS spectra of body fluids via two approaches for this forensics application. In one, the cotton swab is dipped in 50% HAc, lightly rubbed on the dried body fluid stain, then touched to the SERS substrate, and a SERS spectrum is obtained. In the second method, a solution of gold (Au) nanoparticles is pipetted onto the cotton swab, the swab is dipped in the HAc solution, then contacted with the suspected body fluid stain before the swab is directly placed at the focus of the Raman instrument for signal acquisition. High-quality SERS spectra are obtained with both approaches for easy and rapid practical use.

In addition, this presentation shows that the signature of these body fluids is unaffected by the surfaces they are found on. For example, SERS spectra of (1 $\mu$ L) dried blood on nine different materials have been acquired by the Au particle-covered swab technique and result in identical blood SERS spectra. Finally, a number of lab synthesized and commercial Au and silver (Ag) substrates as well as portable Raman instruments capable of rapid SERS use were tested, and an optimized choice for this forensic purpose will be described.

In conclusion, a portable SERS-based methodology has been developed offering a single-platform for confirmatory identification of human body fluids at crime scenes and in forensic lab settings with higher sensitivity and specificity and faster than techniques currently commercially available to crime scene forensic investigators.

#### **SERS, Body Fluids, Identification**

### B43 A Comparison of Short Tandem Repeat (STR) Allelic Recovery Post-Ultraviolet (UV) Damage Utilizing the PreCR<sup>®</sup> Repair Method: Singleplex Versus Multiplex Polymerase Chain Reaction (PCR) Amplification

Morgan Barrett, BS\*, East Haven, CT 06512; David San Pietro, PhD, University of New Haven, West Haven, CT 06516

**Learning Overview:** After attending this presentation, attendees will gain an understanding of how UV, hydrolytic, and oxidative damage affects the Deoxyribonucleic Acid (DNA) sequence and therefore the STR profile, how the PreCR<sup>®</sup> Repair Mix functions to repair those damages, and whether or not singleplex amplification may be more efficient in STR allele recovery than the multiplex method.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of the nature of the multiplex amplification method and how it relates to UV DNA damage and the PreCR<sup>®</sup> repair process.

DNA is subject to many environmental factors that cause various types of deleterious damage to the molecule, including oxidative, hydrolytic, and UV light-induced.<sup>1</sup> This is a major issue in forensic DNA profiling as forensic samples are often found in a damaged or degraded state. The damage inflicted on biological samples results in a degraded DNA template to be used in PCR amplification and ultimately causes the loss of STR peak signals in forensic DNA profiling.<sup>2</sup> The PreCR<sup>®</sup> Repair Mix by New England BioLabs<sup>®</sup> is composed of enzymes used in the cellular mechanisms of DNA repair.<sup>3</sup> Studies have shown the ability of this mix to repair most types of DNA damage prior to PCR amplification of the core STR loci.<sup>4</sup> However, this repair process does not always result in complete STR profile recovery. Standard STR analysis kits are designed as multiplex PCR amplification reactions, consisting of forward and reverse primers for multiple STR loci. The purpose of this study was to determine whether or not singleplex amplification reactions post-UV damage repaired with PreCR<sup>®</sup> would result in greater STR allele recovery with the idea that singleplex reactions would allow for less competition between individual STR loci primers and primer binding sites, allowing for more efficient amplification.

As larger STR markers tend to be more susceptible to environmental damages, as observed by the “ski slope effect,” this study focused on three STR loci of differing lengths, all with simple, tetrameric repeats for the singleplex amplifications: TH01, D18S51, and CSF1PO.<sup>5,6</sup> Primer sequences for these loci were obtained from STRbase and all forward primers were developed to include a 6-FAM fluorescent tag.<sup>6</sup> Multiplex and singleplex amplifications were performed in triplicate under three different conditions; No Damage, No Repair (NDNR), Damage, No Repair (DNR), and Damage, Repair (DR). Additionally, two damaging events were performed as the consistency of UV damage to a particular sequence is unknown.<sup>2</sup>

Many observations were made with the data obtained in this study. These include the interpretations of any differences in peak height percent recovery post-PreCR<sup>®</sup> repair within each locus between multiplex and singleplex amplifications, any consistencies or inconsistencies between the two damage events, any allele size shift, alterations in peak height ratios, or introduction or disappearance of non-specific peaks, and differences in peak height percent recoveries post-repair between shorter and longer loci and homozygous and heterozygous loci. Further studies that focus on more STR loci, both heterozygous and homozygous, as well as a larger sample size and more damage events are warranted.

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#### DNA Damage, DNA Repair, STR Profiling



### **B44 A Comparison of Genotyping Success of Cotton and FLOQ™ Swabs on Casings Using Direct Polymerase Chain Reaction (PCR)**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the differences in genotyping success rates of different swab materials in direct PCR reactions used to obtain DNA from brass casings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by assisting labs in the determination of how best to obtain and analyze DNA from brass casings and similar substrates.

Often, casings recovered from crime scenes involving firearms will have potential touch DNA evidence of the perpetrator or owner deposited on them. Traditional methods of DNA processing are time-consuming and expensive and rarely generate successful and reliable profiles from touch DNA samples.<sup>1-3</sup> One method to overcome this obstacle is to subject the substrate or swab directly to an amplification reaction, through a process known as direct PCR. Direct PCR has been shown to successfully yield DNA profiles from casings.<sup>4</sup> However, genotyping efficiency among different swab types on unfired casings using direct PCR has not been examined.

In this study, swabs composed of different materials were compared to determine which performed better when direct PCR was used on mock 9mm brass firearm cartridge samples. For the study, 15 9mm brass cartridges were sterilized under ultraviolet light for a minimum of 24 hours. After sterilization, 1µL of female control DNA (9947A diluted to 1ng/µL) was added to each cartridge case and allowed to dry. One set of five cartridges was swabbed using COPAN® microFLOQ™ Direct swabs, following the manufacturer's collection protocol. The second group of five cartridges were swabbed using COPAN® 4N6FLOQSwabs™, according to the manufacturer's protocol. The final five cartridges were swabbed using traditional cotton-tipped applicators following the same protocol as the 4N6FLOQSwab™ samples. The entire swab portion of the microFLOQ™ Direct swabs, and 3mm cuttings of the 4N6FLOQSwabs™ and cotton applicators were added to PCR reactions. All samples were amplified using the Identifier™ Plus PCR Amplification Kit and genotyped on the 310 Genetic Analyzer using GeneMapper™ V 3.2.1 software. None of the samples resulted in complete DNA profiles. Most samples recovered no allele peaks, with only six samples successfully detecting 3–9% of concordant alleles.

Though microFLOQ™ Direct swabs were expected to outperform the other swab types, their small surface area may not have recovered enough DNA to overcome copper inhibition. This study suggests that swab type is not the most important factor in determining genotyping efficiency from these types of samples. Instead, future studies should examine ways to decrease copper inhibition in order to increase the amount of DNA recovered and amplified from brass casings before determining if a difference in genotyping success exists among different swab materials.

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#### **Direct PCR, Swab Types, Casings**



### **B45 A Comparison of the Overall Quality and Quantity of DNA Evidence From Fingerprints Collected From Various Substrates Found at Simulated Crime Scenes**

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**Learning Overview:** The goal of this presentation is to discuss the effect of the type of substrate on the amount of DNA recovered from fingerprints. Following this presentation, attendees will better understand how the surface texture and characteristics can affect the DNA recovery from various substrates

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to a growing body of knowledge regarding the utility of DNA recovered from fingerprints from different substrates.

Latent fingerprints are biometric features representing one of the most common evidence types that can be used for human identification at a crime scene.<sup>1</sup> The selection of an enhancement technique for visualization of fingerprints depends on a number of factors, including the type of substrate and the location or environment of the fingerprint.<sup>2</sup> The most common method of enhancement is the use of various types of powders (e.g., aluminum powder, black magnetic powder, black granular powder, etc.).<sup>3</sup> The powders function by attaching to the components of sweat released by the fingertips, which reveals details of ridge elements, thereby enhancing the visualization of the fingerprints.

This research study was conducted to determine the effects of fingerprint powders on the collection and subsequent analysis of the DNA lifted from different substrates. For collection, fingerprints were placed on six different substrates (steel, aluminum, unpainted wood, painted wood, glass, and faux leather) for a total of 144 fingerprints and left for four different time periods (1 hour, 24 hours, 1 week, and 1 month). Subsequently, half of the fingerprints were dusted with fingerprinting powder and the others left bare. All 144 samples were collected from their respective time periods and the DNA extracted and quantitated using real-time quantitative Polymerase Chain Reaction (qPCR) with subsequent short Tandem Repeat (STR) analysis. It was found that though the fingerprint powder did not interfere with the subsequent analysis, there was a difference in the amount of DNA recovered from different substrates after one month. The qPCR degradation ratio varied from 1.6 (steel) to 7.25 (unpainted wood) and correlated with the percent degradation by the relative DNA absorbance measured at 260nm. To assess the quality of DNA recovered, the extracted DNA was amplified, followed by capillary electrophoresis, and the results correlated with the qPCR results.

The results of this study will aid crime scene analysts by providing information regarding the most desirable locations to retrieve DNA from fingerprints at a given crime scene.

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#### **Fingerprint Analysis, Crime Scene Investigation, Substrates**



### B46 An Internal Validation of the ANDE® 6C Rapid DNA Analysis System for Forensic Samples

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**Learning Overview:** After attending this presentation, attendees will understand the results of the internal validation studies and suitable sample types for the ANDE® 6C Rapid DNA Analysis System.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a thorough validation of a commercially available Rapid DNA system so laboratories can better evaluate their options when considering the implementation of this technology.

In forensic DNA analysis, highly variable regions known as short tandem repeats are tested to generate a DNA profile. The traditional processing of DNA samples is performed in a series of five steps: extraction, quantification, amplification, capillary electrophoresis, and data analysis. While there have been some technological advancements, the traditional DNA lab process can take two or more days to complete. The implementation of Rapid DNA technology in the forensic DNA laboratory has the potential to significantly reduce the time needed to process and generate a DNA profile.

Rapid DNA is the term used to describe the generation of a DNA profile, without human intervention, in less than two hours.<sup>1</sup> Rapid DNA is being incorporated into law enforcement booking stations as well as accredited forensic DNA laboratories.<sup>1</sup> Other applications that can benefit from the use of Rapid DNA include: human trafficking, disaster victim identification, immigration, military applications, and homeland security.<sup>2</sup> Although only recently accepted by the Combined DNA Index System (CODIS) in 2018, Rapid DNA instrumentation first became available as a commercial product in the early 2010s. Due to the wide availability of Rapid DNA instrumentation and the multitude of potential applications, there is a need for forensic DNA laboratories to develop a Rapid DNA infrastructure to ensure proper handling and processing of forensic DNA samples for CODIS entry. A strong foundation will not only continue to expand the CODIS database, but potentially lead to the generation of investigative leads in a timelier manner.

An internal validation of the ANDE® 6C Rapid DNA Analysis System was performed at the Palm Beach County Sheriff's Office to demonstrate reliability and robustness of the instrument. The ANDE® 6C Rapid DNA Analysis System is a fully automated instrument, capable of generating a DNA profile in less than two hours with minimal human intervention. The system uses the FlexPlex27 multiplex assay that includes 23 autosomal loci (D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, D6S1043, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, SE33, D22S1045, and FGA), three Y-chromosomal loci (DYS391, DYS576, and DYS570), and amelogenin.<sup>3</sup> The ANDE® 6C Rapid DNA Analysis System is approved for use at the National DNA Index System (NDIS) by an accredited forensic DNA laboratory for reference sample buccal swabs.<sup>1</sup>

A series of studies were conducted on the Arrestee Chip (A-Chip) to define the performance and limitations of the instrument by evaluating contamination, sensitivity, reproducibility, repeatability, National Institute of Standards and Technology (NIST) traceability, mixtures, and known/mock sample types. All samples run on the ANDE® were processed via the laboratory's conventional typing methods and compared for concordance. Samples processed on the A-Chip include buccal samples from a variety of donors, collected at different timeframes, ranging from fresh to approximately eight years of age.

The results indicate that this technology has the potential to expedite the processing of select DNA samples resulting in a faster turnaround of preliminary associations to aid investigations.

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3. ANDE® 6C Rapid DNA Analysis System Product User Manual. Part Number A0120001014 Rev E, February 2018.

#### ANDE®, Rapid DNA, Validation



### **B47 Accelerating DNA Extractions Using a Microwave Toward Increasing the Speed and Success of Rapid DNA Analysis**

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**Learning Overview:** After attending this presentation, attendees will better understand how a microwave can be used to help extract DNA for Rapid DNA analyses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees how the use of a microwave to extract DNA can greatly increase the speed of extractions and has the potential to also inactivate downstream Polymerase Chain Reaction (PCR) inhibitors.

The goal of this project is to develop a quick microwave-based extraction technique as a front end for rapid DNA analysis. The recent development of rapid and microfluidic technology has made it possible to perform field-able analysis of saliva samples from suspects.<sup>1</sup> However, when confronted with forensic samples such as blood, semen, and touch DNA, these same instruments often perform less well. Currently available instruments can perform DNA typing from extraction through genotyping in fewer than 90 minutes and this newly developed ultra-rapid DNA system can yield genotypes in fewer than 15 minutes.<sup>2,3</sup>

The problem is that the processes these instruments use to isolate DNA are slow and inefficient when utilized with samples other than saliva and may not be fast enough nor effective enough for certain sample types and critical time-sensitive intelligence applications. Results from studies involving crime scene samples indicate the need to mitigate issues with sensitivity and PCR inhibition and to improve the speed of rapid DNA systems. Furthermore, processing difficult samples in remote locations is important for rapid on-site intelligence applications. Thus, there is a need to develop a rapid and efficient front end for processing difficult samples in remote locations. This project will develop microwave DNA extraction methods as a front end to a variety of commercial- and laboratory-based rapid Short Tandem Repeat (STR) genotyping systems to improve the speed and quality of results for rapid genotyping of challenging forensic samples.

The importance of effective forensic DNA extraction methods was reviewed by Lee and Shewale, and while there are many effective procedures, several are time-consuming, use hazardous chemicals, unstable chemistries, and/or are generally not amenable to field deployment.<sup>4</sup>

Microwave DNA extraction methods were first published for gram positive bacteria in 1991 and for eukaryotic organisms in 1993.<sup>5,6</sup> Microwave extraction has been applied to paraffin-embedded tissues, sludge, cyanobacteria, spores human disease vectors, bone, serum, and blood resulting in DNA suitable for both Polymerase Chain Reaction (PCR) and Massively Parallel Sequencing (MPS) applications.<sup>7-16</sup> In a study comparing chemical, enzymatic, and microwave extractions, results showed that the microwave method outperformed the other two methods in both the quality and quantity of recovered DNA.<sup>16</sup> This study concluded that the microwave-based procedure is easy, rapid and cost-effective for high-yield isolation of analytical-quality DNA.<sup>17</sup>

Three different microwaves have been successfully used for DNA extraction in the laboratory. In the first study, a dramatic increase in DNA isolation speed was demonstrated using a conventional 400W microwave oven on eukaryotes, including plants, fungi, snake and protists.<sup>6</sup> In a second study, a microwave DNA isolation method was successfully applied to human forensic samples: blood, saliva, semen, fingernails, and hair, in collaboration with Ted Pella Inc using a computer-driven microwave: Ted Pella Inc., Model 3440 at 800W.<sup>18</sup> Two eight-second pulses at full power with an intermittent rest of 20 seconds could be used to isolate DNA from 16 samples in 400µl of extraction buffer simultaneously while only slightly raising the temperature (4–6°C) in less than one minute, resulting in comparable quality and quantity to conventional methods.<sup>18</sup>

A third study tested the compatibility of microwave-extracted DNA with direct rapid PCR. Replicate 15µl saliva samples were microwave extracted, then amplified directly with a rapid two-step PCR protocol for amplification of a seven locus multiplex. Direct STR amplification and detection were achieved in 13 minutes from microwave-extracted saliva samples.

Microwave DNA extraction can increase the speed of extraction and DNA yield, resulting in high-quality DNA suitable for direct rapid PCR protocols. The system has been applied to a variety of single-source human saliva, blood, and epithelial samples. The protocols will be tested on samples deposited on different substrates, samples spiked with inhibitors, and mixtures. Furthermore, a set of mini STRs has been developed for rapid direct PCR of the microwave extracts with amplification in less than nine minutes.

The inclusion of the microwave digestion at the front end of the analytical stream may help to mitigate PCR inhibition, improve the lysing of cells, and increase the overall yield of input DNA for rapid DNA analysis. These positive effects should greatly improve overall speed and success rates using a rapid DNA processing stream for both laboratory and commercial systems.

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## Microwave, DNA, RAPID



### **B48 Considerations for Database Searching With Siblings**

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**Learning Overview:** After attending this presentation, attendees will be aware of the varying statistical results observed in kinship calculations when associations to a missing person found in the Combined DNA Index System (CODIS) include a single parent and sibling assembled into a pedigree tree and how these variations affect the outcomes of database searches.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science and legal communities by highlighting the chance of missing an identification of a missing person when including a sibling reference into a pedigree search in CODIS. Recommendations to address this situation will be provided.

Since the release of CODIS v6.0 in 2008, missing persons searches performed in the software utilize pedigree trees to search for missing family members in the Unidentified Human (Remains) index of the database. First-order relatives are requested to optimize searching of CODIS, including siblings. Typically, the addition of a sibling to a single parent in a pedigree tree is beneficial for calculating kinship statistics such as a Likelihood Ratio (LR) when a genetic association is found in CODIS, as opposed to a parent alone. However, situations have been observed in which the addition of a sibling to a pedigree tree with a single parent (biological father or mother) has substantially reduced the statistical value of a genetic association compared to using the parent alone. This is attributable to the manner in which alleles are passed from parents to their offspring. Because it is possible that even full siblings may share two, one, or (especially) zero alleles in common, the statistical weight of a CODIS association can be negatively affected by the inclusion of the sibling data. The effect of allele sharing between siblings on statistical calculations can therefore mean that a valid association within the CODIS database may be missed due to search threshold requirements.

It is also possible that the submission of a sample purported to be from a full sibling is actually from a half-sibling. The information regarding the half-sibling relationship may be known, but not included in the submitted case information, or the true relationship is not known to the reference sample donor at all. In some scenarios observed, this is not the case. The addition of a full sibling to a pedigree tree has decreased the LR on more than one observed occasion, sometimes so low that the results are not above the lab threshold for reporting. This leads to the possibility that the addition of a full sibling to a pedigree tree in CODIS may actually depress the kinship LR just enough so that it does not meet the level required for reporting, resulting in a true association that is missed during a search of a large database.

Recommended solutions to address this situation will be presented. These solutions may include ignoring the submitted sibling reference sample when searching in the CODIS database. Sometimes, “less may be more” when calculating kinship LRs in missing persons cases wherein family reference samples include a single parent and sibling.

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### **Kinship, Sibling, CODIS**





### **B49 Internal Validation of the Applied Biosystems™ RapidHIT™ ID System Using ACE and INTEL Cartridges**

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the studies performed and results from the validation of the Applied Biosystems™ RapidHIT™ ID System for processing both reference standards and crime scene evidence for forensic casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that this validation will provide a budding resource that can process DNA samples more expediently than conventional processing methods.

Rapid DNA is the accelerated processing of crime scene or reference samples to develop a DNA profile without human intervention in less than two hours. The addition of Rapid DNA technology to a DNA laboratory can significantly advance the workflow, as conventional DNA processing currently requires a minimum of two days to complete, from sample intake through data analysis.

Other areas in law enforcement, such as booking stations, can also benefit from integrating this technology. As the implementation of Rapid DNA expands, the development of a Rapid DNA infrastructure among agencies is critical to ensure the responsible handling and processing of samples for DNA analysis. An established Rapid DNA network will not only continue to supply the CODIS database with DNA profiles, but also expedite the processing of time-sensitive DNA samples and casework.

The Applied Biosystems™ RapidHIT™ ID System is a fully automated instrument capable of taking human DNA samples from sample to profile in approximately 90 minutes.<sup>1</sup> The RapidHIT™ ID System utilizes Short Tandem Repeat (STR) -based identification and review through an incorporated expert system. The instrument accepts two types of sample cartridges: the ACE Cartridge, which is used for single-source reference samples, and the INTEL Cartridge, which is designed for lower template, crime scene evidence samples. Both cartridges use the GlobalFiler™ Express STR typing chemistry for amplification.

The integrated software, RapidLINK™ v1.0, is capable of producing electropherogram results for analyst review using GeneMarker™ HID STR Human Identity Software.<sup>2</sup> The RapidLINK™ software has the capability to link multiple RapidHIT™ ID systems across a shared network, allowing multiple data sites to be managed from one location. This network of instruments allows the user to share and review results in real time and equips the user with a databasing function for samples that are not eligible for CODIS upload.

The validation study conducted for the RapidHIT™ ID System consisted of a contamination assessment, sensitivity, reproducibility, repeatability, National Institute of Standards and Technology (NIST) traceability, mixtures, and known/mock sample types for both the ACE and INTEL Cartridges. All of the validation samples were processed with the accredited laboratory's conventional DNA analysis methods and compared for consistency.

After assessing the RapidHIT™ ID analysis results in tandem with the results generated from traditional forensic DNA methods, it was determined that the instrument is capable of obtaining concordant DNA profiles. Therefore it was concluded that the RapidHIT™ ID System is a reliable tool for the expeditious processing of reference and crime scene samples, allowing for quick inclusions and exclusions with the potential to further criminal investigations.

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2. Applied Biosystems™. RapidLINK™ Software v1.0. *User Guide*. November 2018.

#### **Rapid DNA, RapidHIT™ ID, Validation**

### B50 Performance of Microhaplotype and Short Tandem Repeat (STR) Biomarkers in Mixture Detection and Deconvolution

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**Learning Overview:** After attending this presentation, attendees will understand the use of different biomarkers for mixture detection and deconvolution.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing microhaplotype markers as a supplemental tool to conventional STR typing analysis.

Microhaplotypes (microhaps) are emerging biomarkers defined by two or more closely linked Single Nucleotide Polymorphisms (SNPs) in <300bp displaying multiple allelic combinations.<sup>1</sup> The presence of multiple SNPs within the same locus augments the information over a stand-alone SNP locus. The multi-allelic nature of microhaps, although less polymorphic than STRs, makes these markers a promising versatile toolset as it offers certain advantages over STRs. These are absence of stutter, same-size alleles within a locus, low mutation rate, and ancestry informative alleles. Microhap multiplex assays can reach the same power of discrimination of STRs and also be used for different forensic applications, including human identification, mixture deconvolution, relationship testing, and ancestry prediction.<sup>2</sup> Unlike the standard Sanger sequencing methodology, which does not determine the *cis/trans* relationship among individual SNPs, Massively Parallel Sequencing (MPS) allows determining the parental SNP haplotypes by clonal sequencing of individual amplicons originating from each individual strand, thus distinguishing each parental allele at a locus. This study evaluated the mixture performance of a 74 microhap locus-assay on the Thermo Fisher Scientific™ Ion Torrent S5™ system and compared the results to standard sized-based STR and sequenced-based STR analysis.<sup>3,4</sup>

The detection limit of the bioassay was tested in triplicate using a 2ng to 25pg range of input DNA. The performance of the microhap panel was evaluated in parallel with the GlobalFiler™ kit on Capillary Electrophoresis (CE) on the ABI® 3500 Genetic Analyzer and the Precision ID GlobalFiler™ NGS STR Panel v2 on the S5™ platform. To simulate casework-like DNA samples, a large series of two- to five-person mixtures was simulated at 1-10ng input DNA and at different contribution ratios. Examples of the tested ratios included 10:1 to 40:1 for two-person mixtures, 5:5:1 and 10:5:1 for three-person, 20:5:1:1 for four-person mixtures, and 5:5:1:1:1 for five-person mixtures. For genotyping of microhap loci, Microhaplotyper Plugin v8.1 was used while STR sequence data and CE STR data were analyzed with Converge and GeneMapper® ID-X software, respectively.

The 74-locus panel was found to be sensitive to approximately 50pg input DNA (equivalent of eight diploid cells). Overall the deconvolution and interpretation of CE-based STR mixtures was challenging for imbalanced and high order mixtures. However, sequencing data demonstrates that the deconvolution of these latter can be enhanced by MPS. This approach allows detecting additional minor alleles masked by stutter with conventional CE but distinguishable by sequence and displaying repeat pattern variation or due to the presence of microvariants. It is worth noting that both sequencing of microhap and STR mixtures displayed imbalanced coverage between different loci in all mixtures tested using an input amount of 1–10ng DNA. For microhaps and, in particular, for two-person mixtures, a full minor profile was reported at 1:10 ratio with minimal allele and locus dropout at 20:1, and more significant locus dropout at 40:1 ratio at both 1ng and 10ng DNA inputs. At the same input DNA amount for three- to five-person mixtures, full microhap profiles could be reported for the minor DNA contributors at all tested mixture ratios with the exception of some microhap loci that underperformed.

These findings suggest that microhap biomarkers can enhance the deconvolution of mixed DNA samples and complement both conventional size-based and sequence-based analysis of STRs.

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#### Microhaplotypes and STRs, Mixture Deconvolution, Massively Parallel Sequencing

**B51 Optimization Parameters of Fragmentary Voltage and Collision Energy for the Identification and Separation of 1,3,5-Triamino-2,4,6-Trinitrobenzene (TATB) From Other Explosives With Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)**

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**Learning Overview:** After attending this presentation, attendees will better understand the development and optimization of the parameters for the creation of a new method for the identification and separation of organic explosives.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing the current capabilities of the Kosovo legal science lab in the development of new methods for the identification and separation of organic explosives by chromatographic methods and the impact on the identification of source(s) explosives and interconnections to identify terrorists.

Explosives are classified in several ways, based on different criteria. Thus, explosives are divided into high and low explosives based on the type and speed of the action. Explosives are also classified according to their chemical structure. The most important group is that of organic compounds containing the nitro (NO<sub>2</sub>) group. They are further subdivided based on the site where NO<sub>2</sub> is attached to the atomic structure. Nitrogen compounds contain C-NO<sub>2</sub> groups, a group of C-O-NO<sub>2</sub> nitrate ethers, and C-N-NO<sub>2</sub> nitrite nitriles.

There are different methods for identifying and separating organic explosives, especially 1,3,5-triamino-2,4,6-trinitrobenzene (TATB), but recently, double-mass spectrometric detector chromatography has begun to accommodate numerous scientific researches by forensic scientists to find appropriate parameters for the identification and separation of organic explosives in low concentrations in picograms (pg) or even in femtograms (fg).

Therefore, considering the needs, reasonable work has begun to create optimal parameters for Collision Energy (CE) and Fragmentary Voltage (FV) for identifying and dividing TATB from other explosives by using LC/MS/MS with the Atmospheric Pressure Chemical Ionization (APCI) model, Zorbox<sup>®</sup> SB-C18 column 600 bar 3 x 50mm 3.5µm and mobile phase methanol/isopropanol/water (1:3:6), as well as 0.1% chloroform.

In studying this, optimal CE and FV values have been established for the identification and separation of TATB from other explosives, the change of fractional energy values greatly influences the fragmentation of the molecule under study, the fragmentation fractions molecule at all levels, and this facilitates the identification of the molecule of the unknown explosive, and all fractions comply with the preliminary knowledge of the TATB structure and knowledge of its potential fragmentation, in addition to the excellent division achieved with this technique and the limit of detection down to fg.

At the end of this study, optimized parameters for identification and separation of organic explosives with LC/MS/MS result in the following conclusions. This study analyzed the following organic explosive samples: 3,4,8,9,12,13-Hexaoxa-1,6-diazabicyclo(4.4.4)tetradecane (HMTD), 1,3,5,7-Tetranitro-1,3,5,7-tetrazocane (HMX), RDX, TATB, 1,2-dinitroxyethane (EGDN), 1,3,5-Trinitrobenzene (1,3,5-TNB), 1,3-Dinitrobenzene (1,3-DNB), *N*-Methyl-*N*-(2,4,6-trinitrophenyl)nitramide (TETRYL), 4-amino-2,6-dinitrotoluene (4A-DNT), Nitrobenzene (NB), Nitroglycerin (NG), 2-amino-2,6-dinitrotoluene (2A-DNT), 2,4,6-Trinitrotoluene (TNT), 2,6-Dinitrotoluene (2,6-DNT), 2,4-Dinitrotoluene (2,4-DNT), 1,3,5-Trinitro-2-[2-(2,4,6-trinitrophenyl)ethenyl]benzene (HNS), 2-Nitrotoluene (2-NT), 4-Nitrotoluene (4-NT), 2,2-Bis[(nitrooxy)methyl]propane-1,3-diy dinitrate (PETN), 3-Nitrotoluene (3-NT), 3,3-Dimethyl-1,2-dioxacyclopropane (TATP), and CARBAMITE. This work allowed the creation of methods for the identification and separation of explosives doing the optimization of parameters on LC and MS/MS.

During the study, methods for specific explosives were created: HMX, RDX, TATB, TETRYL, TNT, 2,6-DNT, HNS, PETN, and CARBAMITE. These were named: (1) EKSPLOSIVES-MMI-APCI.m, and (2) EKSPLOSIVES \_2-6-2-4\_DNT\_MMI-APCI.m.

Parameter optimization for both methods was accomplished as follows: flow of mobile phase was 0.5mL/minute, temperature of column was 35°C, and the length of column was 3x50mm, the diameter was 3.5µm, the Ionization source Multimode (MMI), ionization model (APCI), and volume of sample injected was 20µm. Mobile phase was suitable for both above methods.

Optimization of parameters was conducted as FV and CE for the explosives listed above to identify and separate explosives in a trace amount at the fg level.

**TATB, Collision Energy, LC/MS/MS**



### B52 Forensic Fiber Analysis by Thermal Desorption/Pyrolysis Direct Analysis in Real Time Mass Spectrometry (TD/Py-DART®-MS)

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**Learning Overview:** After attending this presentation, attendees will better understand an alternative method for fiber analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting how TD/Py-DART®-MS could be used to analyze the polymer backbone of the fiber and other chemical residues from different fiber treatments during manufacturing.

The DART®-MS and TD/Py system can be used to distinguish among different types of fibers and provide orthogonal information for fiber identification. This research is a pioneer for gradient heating controlled Py-DART®-MS, which will hold great potential across a broad range of topics in the analytical chemistry area and impact the forensic science community, especially within forensic fiber analysis.

The forensic examiner should perform a combination of methods to characterize the fiber evidence in order to provide a complete and specific description of the item and rigorously assess its uniqueness and value as evidence.<sup>1-3</sup> The identification or the comparison of fibers requires an examiner to perform at least two of the analytical techniques for each of the following categories: generic class, physical characteristics, and color. Morphological comparison of fiber evidence by microscopic techniques is required in forensic case work since it can offer characteristic information of fibers in all three categories. Additional analysis is required for each category and, ideally, orthogonal techniques should be used (inorganic/organic, spectroscopy/chromatography/mass spectrometry, etc.) to provide the most discriminating information.<sup>4-6</sup> Ambient MS methods such as DART®-MS can be an alternative method for fiber analysis. The objectives of this research were: (1) to evaluate the potential of DART®-MS for fiber analysis, and (2) to classify fiber samples based on their types by using DART®-MS technique and chemometrics.

The pyrolysis device, IonRocket®, was coupled with DART®-MS to assist the analysis. The IonRocket® system is an ideal unit for the analysis of polymers with which the samples can be heated up to 600°C with accurate temperature gradient control for DART®-MS analysis. The temperature was increased at the rate of 100°C per minute, so data with three dimensions consisting of  $m/z$ , time (=temperature), and intensity was generated, which further enhanced the discriminating power comparing with sole DART®-MS analysis when chemometrics was applied. TD/Py-DART®-MS was used to analyze fiber evidence efficiently and effectively because it provided an objective evaluation for a greater variety of characteristics of fiber evidence than the optical microscopic method and eliminates the need for complicated sample preparation procedures necessary for other MS-based instrumental analyses. A wide array of information related to the physical properties (e.g., melting point, sublimation point, and degradation temperature) and chemical composition of fiber evidence was achieved from TD/Py-DART®-MS analysis.<sup>7</sup> The versatility of the analysis can increase the discriminating power (specificity) and improve the accuracy of results for fiber evidence while still providing timely results for investigators. Principal component analysis was successfully applied to classify the fibers based on the materials of textile fibers.

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**Fiber, DART®-MS, Chemometrics**



## B53 Preservation of Trace Compounds From Headspace Analysis in Adsorbent Capillaries

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**Learning Overview:** After attending this presentation, attendees will better understand the stability of gasoline-derived compounds captured by dynamic headspace sampling with alumina as the adsorbent. This presentation considers evidence stability as a function of storage conditions and storage interval to address situations in which evidence must be preserved prior to analysis (hours to days) or for re-analysis (weeks to months).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing experimental and statistical methods to quantify the distribution of compounds captured by an adsorbent. These methods may support archival of forensic samples collected by headspace methods, including fire debris extracts, by providing quantitative evaluation of chromatogram reproducibility.

Headspace vapors are collected to extract trace compounds from complex solid matrices (e.g., fire debris) or in other situations where the solid or liquid under investigation cannot be directly analyzed. Static headspace sampling is appropriate for volatile compounds; headspace concentration must be employed for low-volatility compounds. The National Institute of Standards and Technology (NIST) has developed a dynamic headspace sampling method that utilizes alumina Porous Layer Open Tubular (PLOT) capillaries to concentrate headspace vapors. In this method, an inert carrier gas sweeps headspace vapors through the capillary, which can be cooled if needed, depending on the volatility or stability of the analyte(s), to promote adsorption. The capillaries can be eluted with solvent and analyzed by any analytical technique, typically Gas Chromatography with Mass Spectrometry (GC/MS).

PLOT dynamic headspace sampling was originally developed to identify compounds in the headspace of low-volatility explosives and has been successfully applied to the detection of gravesoil, among many other applications.<sup>1,2</sup> PLOT dynamic headspace sampling can be performed in the field with a portable briefcase-sized unit. The method detection limit has been determined for diesel fuel, and explosives- and gravesoil-related compounds have been detected inside a simulated shipping container.<sup>3,4</sup>

This report investigates the distribution of compounds recovered from alumina capillaries after storage in heat-sealed polyester/polyethylene laminate evidence bags. To generate large populations of nominally identical samples, individual headspace vials containing a known liquid sample were sampled with individual capillaries. The liquid sample was 50% weathered gasoline, which contains a variety of volatile compounds ranging from C<sub>7</sub> (e.g., toluene) to C<sub>11</sub> (e.g., methylnaphthalene). The headspace vial was equilibrated in an oven at 60°C, and the cryostat containing the “trap” portion of the capillary was cooled to 0°C. Headspace vials were purged with carrier gas at 0.6mL/min, and the total collection volume was 1.5mL. Capillaries were heat sealed in evidence bags and randomly assigned to room temperature storage or refrigeration and to various storage intervals.

GC/MS analysis included total ion chromatograms and selected ion monitoring (*m/z*) for alkanes (57, 71, 85, 99), cycloalkanes (55, 69, 83), aromatics (91, 105, 119), indanes (117, 118, 131, 132), and polynuclear aromatics (128, 142, 156). Principal component analysis was applied to probe differences between time points and among samples from the same time point. The analyses investigated variations in the carbon number, carbon class, and total mass of retained compounds. Individual compounds were also monitored. These statistical methods quantitatively demonstrate the reproducibility of the collection method; however, after six weeks, room temperature storage leads to the loss of low molecular weight compounds. Furthermore, the samples lost compounds in a divergent manner. The methods described here can be used to evaluate any adsorbent material stored for any time period and should be applied to situations in which vapor samples must be collected in a field setting and preserved until analysis.

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### Fire Debris Analysis, Evidence Stability, Principal Component Analysis

## B54 The Sensitivity of Fingermark Color Contrast Between Sexes

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**Learning Overview:** After attending this presentation, attendees will be familiar with the use of image color contrast as a tool to distinguish latent fingermark aging patterns of males and females in a monitored, indoor environment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the robustness and sensitivity of using color contrast as a potential age estimation model for latent fingermarks.

For decades, latent fingermark identifications have been routinely performed to link perpetrators and victims to crime scenes and pieces of evidence. However, a reliable and validated method for determining the time of deposition of a latent fingermark has yet to be standardized. This valuable piece of information could help corroborate witness statements and possibly exclude individuals from being present during the occurrence of a crime. Researchers have started to address this matter by investigating the degradation patterns of latent fingermarks in various environments.

Prior research has identified four morphometric parameters that could be used to quantify visual changes that occur during the aging process of latent fingermarks, one of which is the color contrast between the ridges and furrows after enhancement with a powder.<sup>1-6</sup> The methods used to analyze the morphometric parameters were inexpensive, readily available, and easy to perform, which is crucial for implementation into routine forensic casework. Previous results showed that the environmental factors of substrate type (glass vs. plastic), secretion type (eccrine-rich vs. sebaceous-rich), and natural lighting condition (light, penumbra, vs. dark) influenced latent fingermark degradation differently.<sup>1-6</sup> To better understand the observed trends, additional factors must be examined in combination with the aforementioned environmental conditions, including human factors such as biological sex. The purpose of the present study was to determine whether latent fingermarks from males and females have distinct aging processes that lead to significant differences in changes to the color contrast parameter over time.

The current experiment involved aging a total of 756 sebaceous-rich latent fingermarks from seven males and seven females over a period of three months. Inclusion and exclusion criteria were used to minimize exogenous factors and other sources of human variation. The impressions were deposited in triplicate on glass and plastic substrates using a controlled amount of pressure and contact time. These were stored in complete darkness in an environment in which the temperature and humidity were continuously monitored. At nine discrete times, random impressions on glass and plastic for each individual were enhanced with titanium-dioxide powder, photographed, edited, and analyzed using digital imaging software. The image color contrast data in grayscale was collected from histograms that display the distribution of every pixel color making up a photograph. Darker pixels were represented by low color values (with zero being pure black), while lighter pixels were represented by high color values (with 255 being pure white).

Preliminary data revealed an increasing trend in the mean pixel color value of the images over the nine aging periods and a decreasing trend in the amplitude pixel color value. The mean is the average of all pixel colors contributing to the color in the image while the amplitude is a measure of how many different colors are making up the image. The trends in these values were observed in the impressions on both the glass and plastic substrates with no observable differences between the males and females. As the latent fingermarks aged, the powder appeared to be more dispersed throughout the impressions. This shifted the histograms toward the lighter pixel colors causing a higher mean value, a lower amplitude value, and a more concentrated distribution in the histogram (Figure 1).

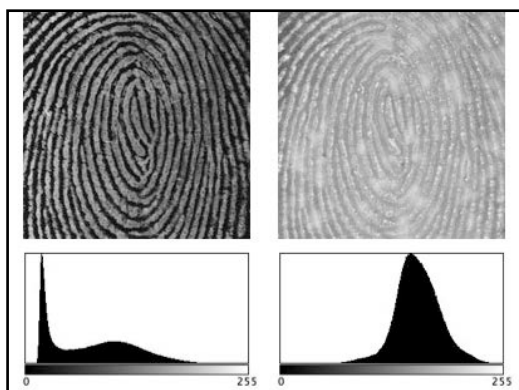


Figure 1. Examples of a fresh (left) and a three-month-old (right) enhanced latent fingermark on glass from a single male and their representative histograms. The ridges are shown in white.



The results of this study suggest that male and female latent fingerprints have similar aging patterns and comparable trends in changes to the image color contrast over time. From a practical perspective, an age estimation model based on color contrast could therefore be robust enough to include impressions from both biological sexes but sensitive enough to detect degradation over time. This study contributes to the current knowledge base regarding visual aging parameters of latent fingerprints and provides a foundation for future research on the influence of other human factors.

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### Biological Sex, Aging, Color Contrast



### B55 Data Fusion From Spectroscopic Techniques for the Discrimination of Colored Automotive Paint Mixtures

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**Learning Overview:** After attending this presentation, attendees will be better informed regarding the importance of how to combine analytical data from two different spectroscopic methods when applied in the context of automotive paint examinations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community, in particular trace evidence examiners, by exploring the potential to properly carry out data fusion to obtain data sets of discriminating features in light of addressing interpretive aspects (i.e., source attribution questions) following comparative examinations of paint specimens.

Paint evidence examinations are conducted following an analytical sequence that typically starts with microscopical examinations, followed by methods of instrumental analysis. While comparing sets of questioned and reference items, data obtained from the various techniques are usually evaluated sequentially, in the sense that when differences are noted between compared sets, there is no need to proceed with further testing. In cases in which the forensic scientist cannot differentiate the compared sets, a subsequent method of the adopted sequence is applied. When the compared sets are indistinguishable at the end of the chosen sequence, an overall evaluation of the data is made to address the question of a common source. The rarity factor of the different features measured with the various methods in a population of interest is of utmost importance to address the “source” question: estimating the chances to observe another coated object in the relevant population that exhibits the same properties as those observed within the questioned specimens as a function, for example, the observed microscopic features *and* infrared profile *and* elemental profile. The present study focuses on the method of combining data obtained from different techniques, where the measured features are typically known to be dependent. The confined scope of this study is to investigate whether the method of combining data from two analytical techniques may affect the outcomes of data analysis for grouping or classification purposes. The way to combine data from different methods is considered critical. These measurements are not carried out on the same area of the specimen simultaneously; hence, it is hypothesized that this process has a greater variability than if measurements using multiple methods would be collected simultaneously.

Automotive repair paint samples collected from auto body shops were mixed at known proportions. Three sets of mixtures were created from the following pairs of base colors: yellow and blue; yellow and red; and blue and green. Proportions at 70%:30%, 50%:50%, and 30%:70% by weight were prepared. Reflectance visible Microspectrophotometry (MSP) (spectral range 400–700nm) and micro Raman spectroscopy using a near-infrared laser source at 785nm (spectral range 2,000–250cm<sup>-1</sup>) were used. Seven replicates of the base color paint and the three mixtures were collected for both techniques. These two methods were selected because of the assumed dependencies between the collected data since both techniques inform about the colorant components. Raman spectroscopy allowed identifying the monoclinic polymorph of bismuth vanadate (BiVO<sub>4</sub>) or C.I. Pigment Yellow 184 for the yellow base paint, the blue phthalocyanine or C.I. Pigment Blue 15 for the blue base paint, the green phthalocyanine or C.I. Pigment Green 36 for the green base paint, and the Diketo-Pyrrolo-Pyrrol (DPP) or C.I. Pigment Red 254 for the red base paint. The classic data analysis procedure of Principal Components Analysis (PCA) followed by Linear Discriminant Analysis (LDA) was used. LDA was conducted on a derived data set consisting of the three principal components that cover about 95% of the total variance of each data set. Data analysis was carried out on the MSP data set, the Raman data set, and a combination of data from these two methods when measurements 1 to *m* of sample 1 to *n* using MSP are combined sequentially side-by-side with measurements 1 to *m* of sample 1 to *n* using Raman. Four additional data sets were created by randomly pairing MSP and Raman data. For LDA, the data sets were randomly split in training sets of four samples and in testing sets of the three remaining samples.

Overall, the accuracies of the PCA-LDA models vary between the side-by-side combinations and those obtained randomly. Low accuracy values were observed; MSP was not sensitive to the color nuances resulting from mixing the paint components, and the pigments detected using Raman underwent known selective resonance effects that would enhance Raman bands from one particular pigment, irrespective of the mixed proportions.

#### Trace Evidence, Automotive Paint, Data Fusion



### B56 Forensic Discrimination of Concrete Pieces From Different Sources Using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS)

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**Learning Overview:** After attending this presentation, attendees will understand what type of elements are in the acid-soluble components of concrete and the variation in their concentrations when they are from different sources. Attendees will also be shown how to calculate indicators of discrimination based on the concentration of those elements and how to use those indicators to identify differences in concrete.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a new method of discriminating concrete based on elemental profiles of concrete pieces as physical evidence.

Because concrete is a familiar material used for building structures and block walls, concrete fragments produced in relation to crimes can be important physical evidence. Criminal investigators sometimes have to evaluate the similarity of fragmented concrete samples in order to determine whether they derive from the same origin. Generally, such samples are examined to identify whether they match certain physical structures. In that process, subjective observation should be minimized, because the results may vary from one inspector to another. For those reasons, the forensic discrimination of concrete should refer to objective indicators measured during instrumental analyses, to which end a method focusing on contained trace elements can be effective.

In this study, the components of cement and concrete that are soluble in nitric acid were subjected to ICP/MS. Indices expressed as the ratios of concentrations for selected elements were calculated from the results, and discrimination was performed based on the difference between the intra-sample and inter-sample variations of those indices.

The qualitative analysis of an acid-soluble fraction prepared from a cement sample confirmed that the fraction contained copper (Cu), zinc (Zn), rubidium (Rb), strontium (Sr), zirconium (Zr), barium (Ba), lanthanum (La), cerium (Ce), neodymium (Nd), and lead (Pb). Because the same elements also appeared in another cement sample in the experiment, those elements were used as indicators and quantified. La was selected as the normalizing element due to its relatively high signal intensity and small deviation within the sample. Once differences in the normalized values were detected between the samples, discrimination was performed using those values as indicators.

To discriminate samples, a range of the average value  $\pm 2 SD$  was first calculated for each sample, and ranges between two samples were compared. If the ranges did not overlap, then a sufficient difference between them was identified that, in turn, suggested the possibility of discriminating the samples. If the ranges overlapped, however, then no sufficient difference between the samples was recognized, which suggested that distinguishing them would prove difficult. The discrimination of two samples of interest was performed for all combinations.

When only one indicator was used, many pairs of samples could not be distinguished, and although the discrimination power between the samples was insufficient, it could be improved by combining all nine indicators. If multiple indicators did not overlap the range of  $\pm 2 SD$ , then the samples were considered to be distinguishable. Of 28 pairs from eight concrete pieces, 26 pairs (about 93%) could be differentiated. By combining indices in that way, the discrimination power improved significantly. Nevertheless, it remained difficult to identify differences in concrete blocks purchased from the same retailer, because commercially available concrete blocks are liable to be produced at the same time. Indeed, when analyzing three concrete blocks from the same batch, the samples were not distinguishable from each other. That result indicates not only that the method can be used to distinguish samples, but also that samples from the same origin could be the same.

Because cement can be nearly homogeneous when making concrete, focusing on cement as an acid-soluble component is appropriate for forensically discriminating concrete. Furthermore, because the method used involved comparing ratios, it stands as an excellent approach that does not require determining the dilution ratio of the sample during pretreatment for acid decomposition.

#### Concrete, Forensic Discrimination, ICP/MS



### B57 Black and White Foils as Packaging Material in Murder Cases

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**Learning Overview:** The goal of this presentation is to address the possibilities of highly discriminating multi-technique investigations to obtain information on the background variation for black and white foils that were used in two separate murders. This method has a wider application, however.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by devising and executing a plan for newly encountered materials to make in-depth comparison investigations, obtain information on the meaning of any matches found, and interpret and report this to the courts.

In two separate murder investigations in the Netherlands, bodies were wrapped in polyethylene foils and deposited in canals. The foils were black on one side and white on the other side. Primarily, this type of black-white foil is used in the agricultural sector to control the heat (e.g., as cover on the inside of greenhouses or over the soils where plants are developing).

The secondary use of these foils in the Netherlands is in the illegal cultivation of hemp (e.g., in apartments or in attics, where the black-white foils are used to suppress the heat signature of the plant growth lights so as to hinder detection by investigating authorities).

In the two separate investigations, results for the reference foils in each investigation matched with results for the foils wrapped around the victims in visual investigation (visual aspects such as color, morphology, and layer structure) and chemical composition (Fourier Transform Infrared [FTIR] and Isotope Ratio Mass Spectrometry [IRMS]) characteristics. Although both crimes were apparently drug related, no other tactical link between the two crimes was found.

Since the background variation (variation in these characteristics for visually similar black-white foils, unrelated to these crimes) for the black-white foils was unknown at the time of analysis, 36 reference samples were collected from different sources (foil producers, agricultural applications, and police seizures from illegal hemp growth locations) and information was collected on production, applications, and the distribution network for the primary use in agriculture. Contacts with producers and distributors were established for this project.

Visual and FTIR characteristics were determined for the 36 reference samples. Nineteen reference samples clearly differed in visual aspects, especially in thickness and/or layer structure, from the casework foil samples. IRMS and Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS) characteristics were also determined for the remaining 17 reference samples that did not clearly differ in visual aspects. For IRMS both H and C isotope ratios were measured for the combined foil layers while LA-ICP/MS measurements (42 isotopes ranging from Li7 to U238) were made separately for the black and white layer.

For one of the casework investigations, LA-ICP/MS characteristics were also determined for the casework samples and found to match for the two black-white foils to be compared. Using the combined characteristics (visual, layer structure, IRMS, LA-ICP/MS), two of the reference samples could not be discriminated from the matching casework samples in this investigation.

For the other casework investigation, no LA-ICP/MS analysis was performed on the samples, but even then none of the reference samples matched the casework samples in the combination of the other characteristics.

This presentation will discuss how these results can be used in the interpretation and reporting of the observed matches for the two casework investigations.

#### Black and White Foil, IRMS, LA-ICP/MS



### B58 The Use of Laser-Induced Breakdown Spectroscopy (LIBS) for the Identification of Bullet Holes

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**Learning Overview:** After attending this presentation, attendees will understand the value of LIBS for the identification of key elements present in bullet wipe.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a novel methodology for the identification of bullet holes. It is a rapid and relatively non-destructive analytical method that increases the level of confirmation of bullet wipe identification, thus potentially providing a valuable tool for shooting reconstructions.

Firearms are used in the majority of murder cases and a large percentage of other violent crimes. For many of the investigations related to these events, a shooting reconstruction is completed using trajectory determination. Without first identifying what is and is not a bullet hole at the scene, the reconstruction will not be as forthcoming. In cases where the hole is not obviously from the passage of a bullet, such as if there is an intermediary target, it could be overlooked and left out of the reconstruction. To date, bullet hole identification has been primarily accomplished through chemical tests, such as the sodium rhodizonate and Griess tests. While these tests are efficient in identifying individual chemical elements and functional groups commonly attributed to Gunshot Residue (GSR) and other components present in the bullet wipe (e.g., copper and nickel from a jacketed bullet), they have significant drawbacks. Some of these include the amount of time required to mix and apply the chemicals, the narrow identification of the chemical components of the suspected bullet hole, and the application of chemicals directly to the item of evidence. With the growing backlog of forensic laboratories, a rapid method for bullet hole identification would be of value for the forensic community. This technique must increase throughput and the level of confirmation while also decreasing the destruction of the evidence in question.

In this study, LIBS was evaluated as an elemental analysis method for the identification of bullet wipe. Five common clothing fabrics (T-shirt jersey, sweatshirt jersey, nylon, denim, and fleece) were chosen. These fabrics were shot with two calibers of full copper jacketed ammunition (.22 and .380) at three angles (10°, 45°, and 90°). Five replicates of each sample were analyzed using LIBS from each of the cardinal directions. The resulting bullet holes were analyzed, and the LIBS spectra were examined for the elements lead (Pb), barium (Ba), antimony (Sb), copper (Cu), and nickel (Ni).

Results indicate that this methodology is promising for the identification of these elements using the major non-interfering emission lines for the five elements, which resulted in the analysis of a total of 18 spectral peaks. Pb peaks were completely absent in the control spectra of the fabrics but were present more than 91.33% of the time in the experimental samples. The Ba peaks were absent from four of the five controls (it was detected in nylon) and were present in all the experimental samples. Sb had one peak without spectral interferences, which proved useful for the identification of this element in 96.00% of the samples shot with the .380. However, Sb was not detected on the bullet holes produced with the .22. Cu was able to be identified in more than 98.33% and absent in the controls. Ni was able to be identified in 38.50% of the samples compared to all of the controls, excluding nylon, analyzed at one peak on the spectra and was present in 98.50% of the samples compared to absence in the controls at the other peak examined. There were no observable trends in elements detected based on the bullet's incident angle.

Based upon results obtained in this preliminary study, it was concluded that LIBS has the potential to be a useful method for the identification of bullet holes in fabrics. Future research will include examination of additional calibers and types of ammunition, alternative target materials, determining the capability of LIBS for analyzing bullet holes through bunched or layered materials, and the use of LIBS in conjunction with commonly used colorimetric chemical testing.

#### LIBS, Gunshot Residue, Shooting Reconstruction



### **B59 A Characterization of Nylanthrene Dyes in the Differentiation of Macroscopically Similar Black Fibers Using Light Microscopy and Visible Microspectrophotometry (MSP)**

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**Learning Overview:** After attending this presentation, attendees will better understand the unusual nylanthrene dyes used in the coloration of black polyamide fibers and the abilities of the classical analytical methods of light microscopy and visible MSP to characterize and differentiate them.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community, and, in particular, trace evidence examiners, by discussing the strengths and limitations of the classical analytical sequence of both microscopy and MSP in the examination of fiber samples that appear macroscopically indistinguishable.

While microscopical and instrumental analysis methods can be used to determine the composition of textile fibers recovered in casework, discrimination in the context of comparative examinations is typically valued by examining the color of a fiber. This is just as important when the bulk composition of a fiber is relatively homogenous, as it is with nylon, acrylic, and other man-made fibers, as it is when the bulk composition of a fiber is heterogenous, as it is with cotton, wool, and other natural fibers. However, these types of analyses often come with more than a few difficulties, including very small sample sizes, the need for non-destructive techniques, and the enormous complexity of both archaic and modern dye compositions. Fiber examinations start by means of light microscopy methods. The determination of whether the fibers are natural (e.g., cotton or wool) or man-made (e.g., viscose, polyester, acrylics, or nylons) is initially made. Man-made fibers usually display a large number of morphological features (i.e., width, cross-sectional shape, presence of delustrant particles, or particular surface striations). Polarized light microscopy is used to study optical properties of man-made fibers. Color, dichroism, and fluorescence are important features used during comparative examinations. It is then suggested that either visible MSP followed by Thin Layer Chromatography (TLC) be used if Ultraviolet/Visible (UV/Vis) MSP is not available. While MSP is concerned with the measurement of the color of fiber specimens, typically resulting from dye mixtures, TLC procedures offer the potential to determine the dye class and the number of components of a given mixture. However, what about fiber samples that macroscopically appear identical after examination using tried-and-true methods?

In this project, a reference collection containing 14 black swatches on a shade card was dyed with known concentrations of four known nylanthrene dyes: nylanthrene black GLWC, nylanthrene rubine SBLF, nylanthrene navy LFWG, and nylanthrene orange SLF. These understudied nylanthrene dyes are no longer being used and have since been discontinued, but they are still susceptible to being recovered in casework at the present time. These dyes were typically used for the dyeing of polyamide 6 or 66 and were known for their favorable migration properties, which provided both excellent coverage as well as stain resistance. These fabric swatches appeared macroscopically similar to each other, and each fabric swatch was dyed with either one, two, or three of the four potential dyes with a total dye concentration of 4% in each swatch. Several fibers were isolated from each swatch and characterized microscopically using bright field illumination and Polarizing Light Microscopy (PLM). Cross-comparisons were carried out using the analytical sequence of comparison microscopy and visible MSP. Pairwise comparisons were performed in which a value of one, two, or three was assigned to each compared pair, based on the difficulty of differentiation, with a value of three assigned to those pairs that were easily differentiated, and a value of one assigned to those pairs that were not able to be differentiated. Out of 91 pairs of fibers, 90% or 82 of the pairs could be differentiated. However, 10% or nine pairs could not be differentiated using either comparison microscopy and/or MSP. Of the nine pairs that could not be differentiated, almost half, (44% or four pairs) differed in their concentrations of only navy and black dye concentrations, while 22% or two pairs differed in their concentrations of only black and orange dye concentrations. The remaining 34% or three pairs differed in their concentrations of orange and navy, in their concentrations of black, navy, and orange, and in their concentrations of black, navy, and rubine.

#### **Trace Evidence, Microscopy, Microspectrophotometry**



### **B60 The Forensic Analysis of Skin-Safe Stamp Pad Inks**

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**Learning Overview:** After attending this presentation, attendees will be informed about the potential role of skin-safe stamp pad inks in forensic scenarios, the significant interrogative information that can be obtained from the analysis of these inks, the various analytical methods that can be employed to determine chemical composition, and the results from the analysis of a selection of skin-safe inks currently available on the market.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing analysts with key elementary methods of analysis for skin-safe stamp pad inks. The methods aim to provide results that can improve investigatory efforts for forensic cases involving ink traces of this nature.

Skin-safe stamp pad inks are currently being used at locations such as family entertainment centers, music festivals, adult entertainment, and other venues with the purpose of stamping individuals that visit the location. This is done in an attempt to identify attendees and monitor the number and activity of visitors. The stamps may be customized for the venue, and the stamp pads may be composed of skin-safe inks specifically marketed toward venues that stamp their patrons. The ink composition may be proprietary and that very nature of the inks can effectively render their chemical composition specific to a manufacturer. The detection, analysis, and identification of the chemical composition of these inks could play a significant role in providing crucial investigatory information in forensic cases.

The goal of this research was to scientifically evaluate these inks by documenting the physical and optical properties of the inks both macroscopically and microscopically and to identify the chemical properties of the ink components spectroscopically. The application of Ultraviolet/Visible (UV/Vis) spectroscopy and Fourier Transform Infrared (FTIR) spectroscopy to the analysis of skin-safe stamp pad inks sold for temporary marking purposes has been explored.

Results from this study indicated that each step of the analysis and each technique used by itself was powerful enough to approach closer and closer to a full discrimination of the ink samples and, through successive analytical methods, all of the analyzed samples were successfully discriminated from one another. Furthermore, the identity of the components in the ink samples can be ascertained through the comparison to known standards, such as those found in a spectral library.

The spectral results from this research can be used as reference for potential skin-safe stamp pad ink that may be encountered in a forensic context. The combination of the methods used in the study created a unique array of results that a forensic analyst may use to comment on the nature of the ink traces that may be found on skin. From an investigatory standpoint, such information may be useful in source attribution, determining the movements or locations of an individual, and ultimately aiding in the identification of an individual.

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#### **Ink, Microscopy, Spectroscopy**



### **B61 A Novel Approach to the Identification of Beetles That Colonize Remains: A Chemometric Processing of Direct Analysis in Real-Time Mass Spectrometry (DART®-HRMS) -Derived Chemical Signatures of Carrion Insects**

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**Learning Overview:** After attending this presentation, attendees will better understand the use of DART®-HRMS in the generation of unique insect species-specific metabolome profiles and how statistical analysis can be applied to these profiles to enable insect species identification that is useful for Postmortem Interval (PMI) determination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees about the use of metabolome profiles as unique chemical fingerprints that enable differentiation between species of insects. Attendees will also learn about the application of statistical analysis for processing of insect chemical profiles for differentiation and classification, as well as about methods for handling specimens.

**Hypothesis:** Statistical analysis can be applied to unique metabolome profiles generated by DART®-HRMS of insect-ethanol suspensions in order to accomplish species identification.

**Synopsis:** One of the most important aspects of a death investigation is estimation of time since death, also known as PMI. This can be difficult to assess for remains that have advanced to such a state of decay that traditional methods for determining PMI are no longer applicable. In such cases, carrion insects found on or near the body can assist forensic investigators in accomplishing this task, as there is a well-established correlation between a given stage of decomposition and the insect species that colonize the remains. Since the timeline associated with insect progression through various life stages is well established, knowledge of the species of the retrieved entomological evidence can be used to calculate PMI. For this reason, accurate species identification is critical. However, this process is challenging because different insect species are often morphologically similar at a given life stage. Thus, species identification often requires an experienced entomologist to make a positive identification based on the gross physical features of the adult after rearing the insects to maturity, a time-consuming process. Furthermore, the insects most likely to be found colonizing remains that are in the advanced stages of decay are beetles. Yet, there is a limited amount of research on methods that can be used to quickly identify beetle species.

**Methods and Results:** It is demonstrated here that chemometric processing of DART®-HRMS data acquired from analysis of insects can be used to rapidly accomplish species identification. Five individual dried insect specimens were obtained for each of 18 species of necrophagous insects belonging to the Diptera and Coleoptera orders, such as *Muscidae spp.*, *Necropilia americana*, *Creophilus maxillosus*, and *Nicrophorus tomentosus*. In order to mimic field collection practices, the dried specimens were suspended in aqueous ethanol prior to analysis by DART®-HRMS. The optimal conditions for insect rehydration prior to mass spectral analysis were also investigated. While it was determined that species-specific DART®-HRMS chemical fingerprints could be acquired using dried, non-hydrated samples, consistent results were obtained when dried samples were suspended in aqueous ethanol for at least 24 hours. Suspending the samples for more than 24 hours was found to confer no added advantage, nor was an advantage seen in rehydrating the insects with steam prior to placing them in suspension. The results showed that the ethanol suspensions of each species exhibited a unique chemical fingerprint and that these fingerprints were consistent for members of the same species but different between species. The application of Kernel Discriminate Analysis (KDA) to the data revealed that clear differentiation was possible between insect species based on the chemical fingerprints generated by DART®-HRMS analysis of insect-ethanol suspensions. Furthermore, the separation occurs even between insects belonging to members of the same family, such as the Silphidae family.

**Conclusion:** At the scene of death investigations, insects present on remains are often underutilized as evidence due to the cost, time, and other resources needed to extract the useful information provided by the specimens. The use of DART®-MS and statistical analysis to differentiate between species of insects can circumvent some of these challenges, thereby increasing the value of entomological evidence.

**Forensic Entomology, DART®-HRMS, Chemometrics**



### **B62 A Novel Approach for the Collection and Characterization of Inorganic Gunshot Residue (IGSR) Standards**

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**Learning Overview:** After attending this presentation, attendees will be aware of a novel collection and characterization for authentic IGSR originating from the primer of ammunition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community, which does not have access to an IGSR standard that can be consumed, by attempting to develop one to help advance the discipline of IGSR analysis.

The detection of gunshot residue (GSR) is an important form of trace evidence because it can provide investigative leads, enhance reconstruction, protect citizens from violent criminals, and assist in intelligence operations. Although current methods of Scanning Electron Microscopy/Electron Dispersive X-ray Spectroscopy (SEM/EDS) analysis are scientifically valid, they are time-consuming, typically requiring hours of analysis per sample. Furthermore, studies into new types of rapid instrumentation are slowed by the lack of GSR standard reference materials. A synthetic GSR standard is available for SEM/EDS analysis, but there is no option for a standard that can be manipulated, digested, or deposited onto a substrate. Also, researchers must have firearms and a ballistics lab to test a new method, which can be impossible without access to the proper resources.

A research group at West Virginia University in collaboration with the Sacramento District Attorney's Office—Laboratory of Forensic Services developed a novel method for the collection and characterization of IGSR particles. Ammunition consisting of only a primer and cartridge case was discharged in a controlled environment and suspended in an organic solvent. Then, the particles were collected, stored, and analyzed by three different analytical techniques to characterize the particle's morphology, distribution, and composition of the IGSR standard.

An Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) digestion method was developed and validated for qualitative and quantitative characterization of the IGSR particle suspension. During the preliminary study, the elemental concentration was monitored over three months and an Analysis of Variance (ANOVA) statistical analysis determined the stability of important IGSR markers (e.g., Lead [Pb], Barium [Ba], and Antimony [Sb]) during the time stored. Ruggedness testing was performed to determine the main effects of the digestion method and examined six factors (digestion temperature, digestion time, the time between sample preparation and digestion, the time between digestion and analysis, acid concentration, and analyte concentration) at two levels for 38 different elements. After determining the main effects, the method was validated for the 38 analytes and figures of merit—selectivity, precision, and trueness—were determined.

Since morphology is also a critical aspect of GSR evidence analysis, the standard was assessed to determine if the collection process preserved the spherical morphology and elemental composition typical of GSR particles. The standard was spiked on GSR stubs at multiple time intervals and analyzed by SEM/EDS during the preliminary three-month study. The analysis indicated no degradation of either was observed. To test the homogeneity and reproducibility of the particle deposition, small amounts were spiked onto GSR stubs, and the particles were mapped and counted using an automated GSR recipe and following the American Society for Testing and Materials (ASTM) E1588-17 standard procedure.

Last, laser-induced breakdown spectroscopy was used to test the practical application of the primer standard. The standard was spiked onto GSR stubs and used in a Box-Behnken optimization design and also as a daily Quality Control (QC) sample. The optimized parameters were applied to a micro-chemical mapping method for the detection of IGSR.

A standard such as the one developed can be a powerful tool to greatly enhance the study and interpretation of GSR evidence. For the first time, the forensic community would have access to an IGSR standard of known elemental concentration and number of particles. This study developed a micron-size particle IGSR standard that mimics residues from leaded and non-toxic modern ammunition. The standard is anticipated to strengthen not only future research but also the understanding of the transfer and persistence of GSR by providing ground truth of the number of particles present before activities. A standard can also improve analysis in crime laboratories by providing an option for monitoring the performance of another method of GSR detection besides SEM/EDS analysis. A material such as this can enhance the evidential value of GSR in the long run because the nature of the trace evidence can be evaluated with greater certainty, GSR examiners can provide a clear record of instrument performance, and the community can validate new instrumentation without the challenge of collecting authentic GSR samples.

#### **Gunshot Residue, Standard Development, SEM/EDS**



### B63 Explosive Residue Transfer From Various Explosive Ordinance Disposal (EOD) Render Safe Procedures (RSPs)

*Joseph Stein\*, Richmond, VA 23220*

**Learning Overview:** After attending this presentation, attendees will understand various RSPs used by bomb technicians in the EOD field and how these procedures can potentially transfer explosive residue onto Improvised Explosive Devices (IEDs). Attendees will also learn what specific compounds are found in these residues.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about residues left by some RSPs and how this can affect forensic analysis of IEDs. Examiners will now be aware that residues found on IEDs may have originated from the RSPs and not from the main charge of the device if it was initiated and consumed. This will help them with the interpretation of their results that can ultimately affect the outcome of a case.

Before an IED is sent to a laboratory for analysis, it needs to be rendered safe if it did not already explode or initiate, which can lead to potential contamination of the evidence if residue analysis is important. RSPs include utilizing a Percussion-Actuated Non-electric (PAN) disrupter or a fluid-filled bottle disrupter. PAN disrupters utilize shotgun rounds to propel liquid or solid projectiles that disrupt the electrical fuzing system or break open the container. The fluid-filled bottle disrupter utilizes a plastic bottle that is filled with water and a detonation cord used to disrupt soft containers, such as backpacks.

While the goal of these disrupters is to render the device safe without having it initiate, occasionally the procedure will fail and the IED will detonate. If this occurs, only the residue on the IED fragments can be chemically analyzed to ascertain the identity of the explosive. However, since these RSPs also use explosives, they too can impart residue on the IED. Smokeless powder is used in the PAN disrupter ammunition, which can also be found as a main charge in IEDs. Compounds of analytical interest in smokeless powders include Nitroglycerin (NG), Diphenylamine (DPA), Ethyl Centralite (EC), and Methyl Centralite (MC). The bottle disrupter utilizes a Pentaerythritol Tetranitrate (PETN) -filled detonation cord, another explosive that may be used in IEDs.

Before any testing was conducted, analysis of the bulk powder used in the shotgun ammunition and detonation cord was conducted to determine the explosives and stabilizers present in each. The powders were then burned onto the various substrates to determine if the compounds would decompose after deflagration. To test whether RSPs impart residue on IEDs, the disrupters were fired onto inert containers used in IED construction, such as pipes and backpacks. These were later analyzed for post-blast residue. The PAN disrupter was loaded with two different rounds. A blank was used to shoot a water column at a Polyvinylchloride (PVC) pipe, while a birdshot cartridge was used to target a steel pipe. The water from the bottle disrupter was imparted onto a backpack. All the tests were performed in triplicate.

Instrumentation used in the analysis of the residues included a Gas Chromatograph/Mass Spectrometer (GC/MS), a Liquid Chromatograph/Mass Spectrometer (LC/MS), and a Gas Chromatograph with an Electron Capture Detector (GC/ECD). Analysis of the smokeless powder from both of the shotgun ammunitions used in the PAN disrupter showed the presence of NG, DPA, and EC. Only PETN was identified in the detonation cord used in the bottle disrupter. The residue from the smokeless powder and PETN showed no decomposition of the analytes after they were burned. Two of the three steel pipes rendered safe with the birdshot had detectable amounts of NG and EC. No DPA was detected on any of the steel pipes. None of the PVC pipes had detectable amounts of NG, EC, or DPA. Trace amounts of PETN were detected on two out of the three backpacks. Overall, finding such residue in casework should not rule out the possibility that an individual used a particular explosive in the construction of the IED, but examiners should be aware of residues left by disrupters, especially if the device initiates during the RSP.

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#### Explosives Residue, Render Safe Procedure, Improvised Explosive Device





### B64 Paper-Based Electrochemical Detection of Drugs of Abuse in Sweat

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**Learning Overview:** After attending this presentation, attendees will understand how drugs of abuse can be detected using a paper-based electrochemical sensing method. As paper-based microfluidics is becoming a rapidly developing field, printed electronics is also growing swiftly, especially in the sense of wearable technology. By combining these fields, fluidic samples can be used as substrates for detecting analyte concentrations using voltammetric techniques. This electrochemical drug-detecting sensor is designed for drugs such as opioids to be detected in sweat.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that sweat can be used for detecting the presence of abused drugs through the merger of two practical applications, electrochemistry and paper microfluidics. The design and developmental validations of this sensing method will involve opioid detection in biological fluids, such as sweat.

The use of biological fluids for detecting drugs of abuse has increased rapidly; however, these fluids are typically tested for drug abuse over a short time period. Sweat testing for drugs can take place over a longer time span. Current drug testing using sweat has mainly been used in clinical applications; however, it is uncommon in drug-of-abuse testing. There have been many studies that have facilitated testing procedures for detecting drugs through sweat patch testing. However, the results from the sweat patches were limited due to their varying volumes of perspiration collected onto the sweat patches and the effect of different activities and environmental impact. Using sweat patches to collect sweat for testing provides a non-invasive alternative method of testing a liquid perspiration for opioids and other drugs of abuse.

Sweat is a vital body fluid that can be used to trap and analyze drugs of abuse. Previous studies have shown limitations, such as poor sensitivity and interferences from components in the sweat matrix. Yet, sweat contains drugs and drug metabolites that are present in the body. Therefore, the goal of this presentation is to introduce the development of an application for detecting drugs of abuse, such as opioids and fentanyl, through sweat using electrochemical sensing. Initial work was performed to detect a variety of opiate controls spiked in sweat. This was used to optimize sensor measurements and determine matrix effects. After the optimization of these mixtures, the sensors were further optimized and validated using real samples.

The paper-based devices were prepared utilizing sheets of chromatographic paper and thermal wax to create hydrophilic channels that are bounded by hydrophobic barriers. Electrode patterns were designed using silver chloride (AgCl) and pasted onto the electrode layer of the paper-based device. Upon analysis, the sweat patch allows for drug detection using aptamers in liquid perspiration. Aptamers that target six different opioids were selected and modified for better stability. By covalently linking these modified aptamers with the redox active molecule, changes occur in confirmation, resulting in enhanced signaling for the presences of opiates. The results show the current sensitivity detected, effect of interferences, and whether sweat collected during various activities results in different detection capability.

Overall, this paper-based electrochemical sensing method permits a low-cost, integrative, and cumulative response for the determination of opioids in sweat for the purpose of drug monitoring.

#### Drugs of Abuse, Sweat, Electrochemistry



### B65 Improving the Efficiency of Forensic Science Evaluations of Mass Disaster Damages

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**Learning Overview:** After attending this presentation, attendees will understand that it is possible to estimate and evaluate the financial impacts due to an event. This can result in preliminary responses for complex problems faced by forensic science. A company must recognize on the statement of financial position and the income statement the estimated amounts of liability, and forensic scientists can use this in considering the data bias.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how difficult it is to estimate mass disaster damages.

This presentation seeks to present a relevant study developed after Brazil's Brumadinho dam failure. This disaster involved different areas of forensic science in Brazil.

Three years after the world's largest disaster in the history of mining, in Mariana, Minas Gerais State, Brazil, several government institutions had to work together to answer another dam failure case. On January 25, 2019, a rupture was experienced in the dam of the Córrego do Feijão mine, located in Brumadinho, Minas Gerais, Brazil (named the "Brumadinho dam"). The owner is a private sector, publicly traded company, headquartered in Brazil, and present in several countries around the world. Due to this mass disaster, 270 people lost their lives or are missing. Tons of iron ore waste were contained in the Brumadinho dam. The tailings contained in the dam have caused an impact of around 270km in extension, destroying some of the company's facilities, affecting local communities, and disturbing the environment. An important river and its ecosystems have also been impacted by the event. The company has been trying to mitigate and recover the social and environmental damages resulting from the dam failure. In this context, it is almost impossible to price all costs that may be incurred to restore ecosystems and repair all the damage caused. The detailed statistic results have been consolidated to demonstrate the importance of studying the historical cost recognized for the companies involved, even knowing the restrictions and underestimations employed. Other mass disaster data can be useful when presenting a strong correlation to support the forensic experts' reports.

It is well known that investing in infrastructure is one of the simplest ways of improving the results of forensic sciences. Another important issue is the need for ongoing training and encouraging studies for these professionals. All of these aspects are part of good criminalistics management practices and involve financial and human resources. Sharing experiences, understanding the limits of action of forensic science, and working together can improve the expert's work. It intensifies confidence and makes the institutions stronger. The involvement, articulation, and coordination among government institutions in this study present evidence of significant improvement for forensic results.

#### **Brumadinho's Dam Failure, Forensic Experts Reports, Estimated Costs**



### B66 Front-End Fractionation of DNA and Proteins for the Simultaneous Genetic and Serological Analysis of Sexual Assault Case Samples

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**Learning Overview:** After attending this presentation, attendees will have gained insight into a workflow that permits the fractionation and simultaneous analysis of DNA and proteins in sexual assault samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing comparative data evaluating methodologies for the co-extraction of DNA and proteins. The results of this study demonstrate the compatibility of proteomic workflows and the importance of serological identification in sexual assault evidence testing.

In order to combat the sexual assault kit backlog in the United States, more efficient processes for serological and genetic sample preparation are needed. Currently validated serological methods (with the exception of microscopy) for semen/saliva identification suffer from limitations associated with specificity and sensitivity. By pairing simultaneous fractionation or co-extraction of cellular (DNA for genetic testing) and protein material for use with proteomic serological identification, these limitations associated with traditional antibody-based serological assays are overcome, providing practitioners and investigators with more actionable forensic results. Simultaneous preparation for proteomic and genetic analysis will also aid with expedited turnaround times.

Samples were prepared using single-source vaginal swabs from an individual abstaining from any form of sexual intercourse and single-source semen and saliva from a male donor. Vaginal swabs were solubilized and extract was pooled to normalize protein material. Vaginal extract was applied to clean cotton swabs and fortified with various dilutions of semen and/or saliva prepared in deionized water. Replicate sample preparations ( $n=10$  per method) were co-extracted using one of three evaluated sample fractionation workflows. The first method was a previously validated in-house sample preparation workflow for the proteomic analysis of biological samples in which soaking and centrifugation are used to pellet cellular material for genetic analysis while proteins remain in the supernatant. Protein material underwent a tryptic digestion prior to analysis. A phenol/chloroform organic extraction was utilized prior to genetic analysis. The second co-extraction method developed by Kranes et. al. employed a simultaneous DNA extraction and tryptic protein digestion followed by subsequent fractionation utilizing a Molecular Weight Cutoff (MWCO) filter.<sup>1</sup> The third fractionation method evaluated utilized a commercial product, the QIAGEN® AllPrep® DNA/RNA/Protein Mini Kit, which separates protein and genetic material fractions using selective binding filtration chemistries and protein precipitation. All protein fractions, regardless of front-end prep method employed, were assessed using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS). All DNA extracts were quantified using the Applied Biosystems® Quantifiler® Trio quantification kit, amplified using the Globalfiler™ Polymerase Chain Reaction (PCR) amplification kit, and analyzed on an Applied Biosystems® 3500 Genetic Analyzer.

The three sample fractionation protocols were compared according to the following criteria: (1) peak area intensity observed via UPLC-MS/MS analysis for target protein biomarkers; (2) DNA quantification values; (3) overall quality of genetic profile obtained; (4) consistency among preparation replicates; and (5) speed/cost and ease of workflow. These data demonstrate that sufficient DNA and protein can be obtained from simulated sexual assault samples to allow for the simultaneous forensic analysis of each fraction, eliminating the need for forensic analysts to prioritize one type of testing over another.

#### Reference(s):

- <sup>1</sup> Kranes, S., Sterling, S.A., Mason, K. et al. Simultaneous DNA and protein extraction using trypsin. *Forensic Sci. Int. Genet. Suppl. Ser.* 2017;6:e203–e204.

#### Proteomics, Serology, Genetic Analysis



### B67 Vaping in the Fourth Generation: A Comparison of Nicotine Dose Capture in Different Forms of Concentrated Substances

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**Learning Overview:** After attending this presentation, attendees will be able to understand the use of fourth-generation electronic cigarettes (e-cigs) in illicit drug use and the danger this poses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by further implicating the presence of e-cig use in the realm of controlled substances.

E-cigs were created as an alternative nicotine delivery system. Three generations of traditional e-cigs exist, designed to deliver nicotine through aerosol/vapor by heating an e-liquid solution made up of nicotine, various flavoring agents, propylene glycol, and vegetable glycerin. Two more generations have recently evolved: (1) the fourth generation device, focused on the ability to aerosolize plant material (marijuana), waxes, and dabs (THC based); and the fifth generation “pod mod,” focused on simplicity and ease of use. Novel devices also have more potential to be manipulated for use of other psychoactive substances.

The purpose of this experiment was to determine the efficiency of aerosolizing nicotine in different mediums using a fourth-generation e-cig (the Source Orb 4), which utilizes five different types of atomizers for a more customizable vaping experience.

Two different materials were introduced to each atomizer; a 12mg/mL 50:50 PG:VG nicotine e-liquid and a 12mg/mL nicotine/kava wax mixture. The atomizer was operated at 4.2V, and the generated aerosol was collected in a trap ( $n=5$ ). Nicotine concentrations were determined using a Shimadzu® LC30 Ultra High Pressure Liquid Chromatograph attached to a Shimadzu® LCMS-8050 Triple Quadrupole Liquid Chromatograph Mass Spectrometer (UHPLC-MS/MS). Chromatographic separation was performed on an Agilent® Zorbax® Eclipse XDB-C18, 4.6 x 75mm, 3.5-micron column with 10 $\mu$ L injections. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile, introduced to the column at a 50% binary gradient and a flow rate of 0.5mL/min. The instrument was run in positive Multiple Reaction Monitoring (MRM) mode. The following transition ions ( $m/z$ ) with their corresponding collision energies (eV) in parenthesis for nicotine 163>130 (-23), 163>117 (-24), and nicotine-d<sub>4</sub> 167>134 (-25).

The average percent recovery delivered per puff for the five different atomizers ranged between 31%–80% for the nicotine e-liquid and 23%–189% for the nicotine/kava wax. The measured experimental dose ranged from 0.8–7.4 $\mu$ g for the nicotine e-liquid and 3.7–53.9 $\mu$ g for the nicotine/kava wax.

Depending on the atomizer and type of medium being vaporized, the amount of nicotine aerosolized will vary. The Source Orb 4 was able to weakly aerosolize nicotine in the traditional e-liquid, as compared to the wax. These results further imply the progression of the fourth-generation e-cig in distributing plant and wax material, in a device that has the appearance of a traditional e-cig. As a result, Drugs Other Than Nicotine (DOTNs) with pharmacological and psychoactive properties can be discreetly consumed in public, causing a concern in several aspects of public safety.

*This research was supported by awards from the National Institute of Justice and the National Institute of Health.*

#### Electronic Cigarettes, Nicotine, LC-MS/MS



### **B68 An Evaluation of the Investigator® 26plex QS STR Kit and a Comparison With Two Commercially Available Short Tandem Repeat (STR) Kits**

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**Learning Overview:** This presentation will provide attendees with information regarding the performance of a new STR kit from QIAGEN® that has not yet been marketed in the United States. This project seeks to compare this new kit to two existing forensic STR kits in terms of their sensitivity, resistance to inhibitory compounds, usefulness for casework-type samples, and ability to interpret two-contributor DNA mixtures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about the performance of a new human STR kit compared to two commonly used commercial kits.

STRs are the gold standard in forensic human identification. Several multiplex STR kits are currently on the market, and in recent years, kits have included an increasing number of loci, resulting in profiles with more discriminatory power. Before new kits are implemented in crime laboratories, they go through extensive validation studies. It is important that the chemistries be sensitive enough to produce profiles from low-quantity and low-quality samples. In addition, DNA profiling can be complicated by various Polymerase Chain Reaction (PCR) inhibitors common to forensic sample types, and some kits are better able to handle these inhibitors than others.

In this study, we evaluated the Investigator® 26plex QS kit and compared it to two kits commonly used in forensic laboratories: the Investigator® 24plex QS kit from QIAGEN® and the GlobalFiler™ PCR Amplification kit from Thermo Fisher Scientific™. The Investigator® 26plex QS kit is a new kit that simultaneously amplifies the Combined DNA Index System (CODIS) loci and the European standard loci, plus Penta D, Penta E, D6S1043, DYS391, and amelogenin, along with Quality Sensor (QS) markers to monitor for inhibition. A sensitivity study consisted of varying concentrations of control DNA between 16pg and 2ng. To test the kits' tolerance to common inhibitors, low, medium, and high concentrations of hematin, humic acid, calcium, and collagen were added to control DNA. In addition, a study was carried out to assess the effect of male/female DNA mixtures on profile interpretation. Finally, a variety of casework-type samples were run, including bone, hair, blood, decomposed muscle, Ultraviolet (UV) -damaged, buried bloodstains, and formalin-damaged tissue.

The data show that all three kits produce complete or nearly complete profiles with at least 32pg of DNA, and the Investigator® 24plex QS kit recovers more alleles than the other two kits at a 16pg DNA input. Additionally, the Investigator® 26plex QS kit has a wider dynamic range with relatively clean profiles at a 2ng input, while Investigator® 24plex QS and GlobalFiler™ experienced pullup between dye channels, making the resulting profiles more difficult to interpret. Investigator® 26plex QS and GlobalFiler™ were inhibited by high concentrations of calcium and collagen and experienced significant allele dropout in the presence of these inhibitors. Investigator® 24plex QS was resistant to all inhibitors and only experienced dropout at high collagen concentrations. The genotypes of the shared loci for the casework samples were concordant between the three kits, and the profile quality and completeness were similar. When two-contributor DNA mixtures were assessed, GlobalFiler™ had the most complete profiles for minor contributors. Investigator® 26plex QS and Investigator® 24plex QS preferentially amplified the major contributor alleles, resulting in more drop out of the minor contributor.

Overall, this research investigates and reports on the relative performance of three commercial STR chemistries, including a chemistry not currently available in the United States.

#### **Short Tandem Repeats, Human Identification, Forensic Casework**



### B69 A Non-Destructive Genomic Analysis of Single Pollen Grains

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**Learning Overview:** After attending this presentation, attendees will have gained an appreciation of a non-destructive genomic methodology of single pollen grains that can be complementary to their microscopic analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a simplified, non-destructive procedure for the analysis of every single pollen grain in a sample, which can be utilized in forensic labs at the state or local levels.

With more than 300,000 plant species on the planet, palynology (i.e., the study of the structure and origin of pollen) plays an important role in forensic analysis. Each location has a unique flora at different periods in time, making pollen a perfect proxy to link person(s) or object(s) to a particular place or time. This is the basis of forensic palynology. It relies on the facts that: pollen is an ever-present feature of the environment; different geolocations have different pollen signatures, allowing for inference related to spatial tracking; and plants bloom at different times, allowing for temporal inference. Pollen is extremely durable and can be used for forensic studies decades after sample collection.

Forensic palynology has played a role in a large number of criminal investigations worldwide, including homicide, violent assault, rape, genocide, terrorism, suspected terrorism, and even intelligence on drug smuggling.

The considerable number of potential plant species that would need identification is handicapped by the few ones that are databased using traditional methods, reducing the potential for geolocation. These traditional methods rely on the examiner having a high level of expertise in microscopy and can be time-consuming. With the emergence of DNA barcoding, pollen genomics has an immense potential to classify and identify pollen taxonomically from its genetic signature. This approach characterizes pollen species using a short DNA sequence from a universal standard in the genome and can help transform the current standard vision of forensic palynology by making it readily accessible to a wide range of forensic laboratories and increase the taxonomic resolution of identification. In plants, three regions of the chloroplast genome (matK, rbcL, and trnH-psbA), as well as the nuclear ribosomal ITS2 region have been widely accepted for use as DNA markers, either in combination or separately. Two main advantages of using DNA barcoding in forensics are that: (1) it allows taxonomic identification of parts of the organism that do not display diagnostic morphological characters; and (2) the same method is used across multiple taxonomic groups, so it allows species to be classified without having to involve narrowly defined taxonomic experts.

To date, the most common cleaning process is acetolysis, which involves highly acidic conditions and chemicals that could damage or alter the exine morphology, in addition to destroying the pollen genetic material. Similarly, DNA analysis relies traditionally on the mechanical destruction of the pollen grain to liberate the genetic material. In this work, it is shown that single pollen grain can be examined using universally accepted genetic markers (rbcL, matK, and ITS2) for DNA barcoding using quantitative Polymerase Chain Reaction (qPCR) while keeping their morphological features for microscopy. This is possible by a simple low-temperature treatment of the pollen grain in ethanol, scalable from large quantities down to a single grain. For the first time, DNA and morphological information can be obtained for every single pollen grain in a sample, which can be stored for future examination.

#### DNA, Pollen, Palynology



### **B70 The Enhancement of Human Scent Profiles as Forensic Evidence**

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**Learning Overview:** After attending this presentation, attendees will understand how human odor profiles can be collected and evaluated, why human scent can be utilized as an individual or class characteristic, and why there is a need for profile enhancement of odor profiles for all ethnicities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by portraying the reproducibility of findings in previous Caucasian and Hispanic human scent analyses, as well as the novel incorporation of African American odor profiles.

Human scent has been previously defined as a complex mixture of Volatile Organic Compounds (VOC's) detected in the headspace above a scent sample. Humans generate odor from several areas of the body, including the scalp, hair, mouth, hand, axillae, and foot. The analysis of the chemical composition of human scent has enabled scientists to demonstrate variations within these factors (age, gender, and ethnicity) to distinguish between individuals. Due to the novelty of human scent research, human scent evidence has been undervalued in the court of law. However, this type of evidence has significant value when physical evidence is not available at crime scenes. In order to increase the individualization and differentiation power of human scent evidence, this study aims to further investigate the identification of chemical signatures within the hands and axilla of specific ethnicities and genders.

During the study, the axilla of 68 participants were investigated. Upon collection, samples were extracted using Headspace/Solid Phase Micro extraction (HS/SPME) and Liquid-Liquid Extraction (LLE) and analyzed using Gas Chromatography/Mass Spectrometry (GC/MS). The utilization of SPME immediately followed by LLE complements the extraction of semi-volatile and non-volatile compounds, hence filling in the gaps of the compounds that could not be recovered using HS-/PME alone. This ensured that a full VOC profile is obtained, allowing for improved statistical analysis without requiring any additional sample collection. The samples were evaluated statistically via logistic regression to extrapolate data unique to specific individuals and groups.

Scientific advances have enabled the forensic science community to use scent as a feature for individual or class characteristic determination. The analyses of body odors using the VOCs emitted have proven that, when enhanced, human scent can be as useful as fingerprints and DNA in the attempt to identify individuals. In future work, the VOCs emitted from the axilla of human subjects can potentially correlate to specific Human Leukocyte Antigen (HLA) alleles. Additionally, once the unique odor profiles of each ethnicity can be identified and reproduced efficiently, live human scent training aids for canines can be improved.

#### **Volatile Organic Compounds (VOCs), Solid Phase Microextraction (SPME), Human Scent**



### B71 Bondage Practices in an Asphyxiation Death: An Accidental Death

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**Learning Overview:** After attending this presentation, attendees will better understand the features of a suicidal hanging death that occurred during autoerotic practices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the role of medical-legal investigations in hanging death cases presenting with a mask and multiple ropes and knots, confirming the importance of a multidisciplinary approach for the resolution of cases of forensic interest.

Autoerotic death is an uncommon cause of death with higher prevalence in middle-aged Caucasian people, usually caused by accidental self-hanging. Limiting the flow of oxygen to the brain leads to the accumulation of carbon dioxide, which can induce feelings of euphoria, confusion, and vertigo, which contribute to intensifying orgasm. It is considered by the medical community to be an extremely risky and potentially deadly practice, even for experienced individuals.<sup>1-4</sup>

**The Case:** After numerous reports from some neighbors complaining about nauseating smells coming from an apartment, the corpse of a 34-year-old man was found hanging inside the locked apartment and the body was in an advanced state of putrefaction. A green mask with multiple conical studs was covering his face, his hands were tied with a rope in front of his neck, another rope was encircling his neck and fixed to the lower end of a door, the door handle was covered with a rag; and his feet were touching the floor. After conducting various and relevant examinations at the place of discovery, the body was taken for forensic investigations. Before performing the autopsy, a Computed Tomography (CT) dual scan detection was performed.

**Results:** The CT scan showed the anterior displacement of the hyoid bone, and no evidence of traumatic fractures were documented by the CT imaging. The autopsy findings were: tongue protrusion between clenched teeth; after removing the rope around the neck, a furrow with vital signs and an hemorrhagic infiltrate of the soft tissues and muscles under it were found. The histological findings confirmed the presence of vital signs in the furrow; but not much else because of the advanced state of putrefaction of the body. The study of the knots' disposition defined the death as a self-ligation procedure of the ropes.

In conclusion, the radiological imaging according to the autopsy, histological and toxicological findings, combined with police information, ascribed the death to mechanical asphyxiation occurring during autoerotic maneuvers. According to the witnesses, the man was used to performing self and heteroerotic practices using ropes and masks. The case was solved as an accidental death.

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#### Bondage, Hanging, Forensic Pathology



### **B72 The Characterization of Volatile Organic Compounds (VOCs) Present in the Headspace of Decomposing Livestock, Fish, and Human Remains**

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**Learning Overview:** The goal of this presentation is to present the VOCs between humans and animals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community via the examination of this case study.

Locating human graves is important for crime-solving and archaeological purposes, and the development of tools and methods that can expedite locating clandestine graves has been of keen interest in the scientific community. The efforts focused on establishing the volatile chemical signature of compounds that could indicate the presence of buried human remains where surface clues are lacking and where the content of a potential grave is unknown.

This study analyzed the VOCs present in the headspace above partially decomposed animal tissue samples and directly compared them with results from decomposed human blood and tissues using established Solid-Phase Microextraction (SPME) and Gas Chromatography/Mass Spectrometry (GC/MS) methods. The source of variation between samples was evaluated using Analysis of Variance (ANOVA). ANOVA can assess whether altering the controlled factor, such as flow rate or material, produces a significant difference in the amount of compound collected compared with the differences found in replicate samples. Specifically, this study employed five class prediction methods: Partial Least Squares Discrimination (PLSD), Support Vector Machine (SVM), Decision Tree (DT), Naïve Bayes, and Neural Network. VOCs present in the headspace of different sample parts (muscle, fat, and skin) each from cows, pigs, and fish were identified and compared with human samples. Although there were compounds common to both non-human and human remains, the VOC signatures of each of the non-human remains differed from those of humans. Four VOC signatures (1 phenyl ethanone, pyridine 3 phenyl-, benzeneethanol, 2-(2 methylpropyl) 3,5 di(1 methylethyl)pyridine) were present in only human tissue samples. In addition to detecting six (pentanoic acid, benzaldehyde, 1 phenyl ethanone, pyridine 3 phenyl-, benzyl alcohol, 2-(2 methylpropyl) 3,5 di(1 methylethyl)pyridine) of ten human-specific compounds, one additional unique VOC (2 nonanone) was recorded from non-human samples that were present in non-human samples (beef, pork and fish).

The best decomposition odor class discrimination outcomes have been achieved by PLSD, SVM, and DT. Identifying VOCs that are unique to humans may be useful to investigate human-specific scent and may eventually lead to an instrument that can detect clandestine human burial sites. Further studies are required to routinely retrieve forensic information that is imprinted in VOCs of human decomposition, such as a variety of parts of the body, including large-scale sampling of decomposed animal and human remains, to support criminal investigation. According to the above methods, the analysis was performed to distinguish between human and non-human from the operating gown of the body used in an autopsy. Testing the gown provided good results.

#### **VOC, Human Remains, GC/MS**



### B73 An Assessment of Elemental Homogeneity in Glass Using Micro-X-Ray Fluorescence Spectroscopy ( $\mu$ -XRF) and Laser-Induced Breakdown Spectroscopy (LIBS)

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of elemental homogeneity within a glass source and insight on the capabilities of XRF and LIBS for the forensic analysis of glass.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by comparing modern instrumentation (Silicon Drift Detectors [SDD] -XRF and LIBS) to traditional instrumentation currently utilized in forensic laboratories.

Glass is an important type of forensic trace evidence that can be encountered at various crime scenes, such as hit-and-runs, homicides, and burglaries. The elemental analysis of glass is useful for the discrimination of glass samples originating from different sources and for the association of glass samples originating from the same source.  $\mu$ -XRF is the most commonly used technique for the elemental analysis of glass in forensic casework. The elemental compositions of the known and questioned samples are compared using a match criterion (range overlap or  $\pm 3\sigma$ ) outlined in the standard method American Society for Testing and Materials (ASTM) E2926. However, the match criteria recommended in ASTM E2926 were selected based on error rates obtained for the analysis of glass samples using older instrumentation equipped with Si(Li) detectors. Modern XRF instruments equipped with SDDs are expected to provide better precision, which may increase the risk of false exclusions. Therefore, this study aims to assess the micro-homogeneity of glass using newer instrumentation to establish an appropriate pairwise comparison criterion that minimizes false exclusion rates.

An alternative to  $\mu$ -XRF is Laser-Induced Breakdown Spectroscopy (LIBS), a relatively new analytical technique. Its rapid analysis times and relative affordability make it an attractive option for the elemental analysis of glass. To date, no studies on micro-homogeneity of glass using LIBS have been published, and no standard method for the analysis of glass using LIBS is available. Thus, a secondary aim of this study is to assess sources of variability when using LIBS for glass analysis and establish an appropriate comparison criterion.

The reference glass material National Institute of Standards and Technology (NIST) 1831 was used to compare the analytical performance of  $\mu$ -XRF and LIBS in terms of limits of detection, limits of quantitation, inter-day variability, and intra-day variability. The performance of the  $\mu$ -XRF-SDD system used in this study was also compared to  $\mu$ -XRF-SiLi detectors' data reported in the ASTM method. A vehicle windshield was used to assess the variability of the elemental composition within a single pane. Each of the inner and outer glass panes was divided into 100 sections. Then, a random number generator was used to select 50 sections within each pane randomly. The glass pane was then broken, and 50 glass fragments were collected from both the inner and outer pane of a single windshield and analyzed using  $\mu$ -XRF and LIBS. Each fragment was measured in replicates from different locations (*LIBS*  $n=4$ ,  *$\mu$ -XRF*  $n=5$ ).

The  $\mu$ -XRF method detected nine elements above the quantitation limit for both windshields (Sodium [Na], Magnesium [Mg], Silicon [Si], Calcium [Ca], Titanium [Ti], Manganese [Mn], Iron [Fe], Strontium [Sr], and Zirconium [Zr]), which were further used for comparison between fragments. The false exclusion rates using the ASTM E2926 match criteria was  $>45\%$  for the inner pane and  $>50\%$  for the outer pane. Using a modified match criterion ( $\pm 3\sigma$ , with a minimum  $\sigma$  equal to 3% of the mean), the false exclusion rates were reduced to 11% and 7% for the inner and outer pane, respectively. The LIBS method detected eight elements in the windshields' glass fragments (Aluminum [Al], Barium [Ba], Ca, Potassium [K], Mg, Na, Si, and Sr) with precision ranging between 1%–15%, depending on the element, emission line, and concentration. The LIBS intra-day variability and inter-day variability was inferior to  $\mu$ -XRF but were reduced by estimating Signal to Noise Ratios ([SNR], signal to background near the peak) for each analyte of interest and then using ratios of SNRs to other elements or emission lines. The overall distribution of elemental composition within each glass pane was observed via heatmaps, box-plots, and Analysis of Variance (ANOVA). For pairwise comparisons, different criteria, including range overlap,  $\pm 3\sigma$ ,  $\pm 4\sigma$  was evaluated using three randomly selected fragments as the known sample and compared to one fragment as the questioned item. False exclusion rates were improved from 60% to 8% depending on the comparison criterion applied, the number of replicate measurements, the number of fragments used for comparison, and the data pre-processing methods.

#### Glass, Forensic Science, Elemental

### **B74 The Identification of the Polymer-Bonded Drugs on the Fabric Surface: A Challenge to the Forensic Drug Analysts**

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**Learning Overview:** After attending this presentation, attendees will have useful learning experience about a unique method of drug concealment adopted by drug smugglers and about the identification of polymer-bonded heroin.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating a new extraction.

Drug trafficking through concealment, by adopting various tactics, has always been a method of choice for drug traffickers worldwide. Due to an increase in global demands for illicit drugs, drug dealers and smugglers are designing and adopting new methods to camouflage these drugs to bypass tight security checks at regional and international borders. The detection and identification of drugs from the skillful tactics of smugglers has always been a challenge for law enforcement agencies, international border security forces, and crime laboratories. This case study reveals a new trend in the smuggling of illicit drugs through their concealment as a fabric coating with finishing polymers before exportation. This study also describes the challenges in detection and identification of polymer-bonded drugs.

Fabric bearing a suspected coating of a finishing chemical was seized from an international traveler by the Federal Investigation Agency of Pakistan. It was submitted for examination of the surface coating for the presence of any explosive or narcotic drugs. The surface morphology of the fabric was examined stereo microscopically and led toward the suspicion that it had some unusual coating on its surface. It was further examined by using Scanning Electron Microscope/Energy Dispersive X-ray Spectroscopy (SEM/EDX), which indicated the presence of an unusual coating different than normal finishing coating on the surface of fabric. The coating material was scraped off by using a spatula and extracted in acetone (30min sonication) for explosives analysis. The extract was screened for explosive material by using color tests. No traces of high- or low-explosive materials like Trinitrotoluene (TNT), Research Development Explosive (RDX), Pentaerythritol Tetranitrate (PETN), black powder, flash powder, or Improvised Explosive Mixture (IEM) were found. It was further analyzed by using Gas Chromatography/Mass Spectrometry (GC/MS) and Fourier Transform Infrared (FTIR), but no explosive material was detected. Instead, GC/MS results revealed the presence of heroin (diacetylmorphine). FTIR spectrum indicated the presence of a polymeric material and did not indicate the presence of heroin; possibly the heroin signals were suppressed due to the excessive amount of polymer.

Keeping in view all these results, the scraping of fabric coating was tested for narcotic drugs by using standard test methods that involve the use of methanol for extraction purposes. Methanol extract of the scraping was screened for the presence of illicit narcotic drugs, but no drug was detected. The standard method was modified by replacing the solvent methanol with acetone and including an additional step of sonication for 30min. Acetone extract presented a positive color test for heroin, and a strong signal of heroin appeared on the chromatogram by using GC/MS. This extraction method acted well to unbind the coated material from the fabric and disentangle the suspicious chemical in the coating material employed on the fabric through physical adhesion.

Awareness of the drug analysis community about this new way of concealing illicit drugs will be quite helpful in analysts' routine work.

#### **Smuggling, Polymer-Coated Drug, Heroin**



### B75 The Influence of DNA Extraction Methods on the Quantity and Quality of Retained Genetic Material

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**Learning Overview:** After attending this presentation, attendees will better understand how to characterize the influence of various DNA extraction methods on the quantity (i.e., concentration) and quality (i.e., strand length) of resulting DNA eluates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how better documentation of the influence of DNA extraction and purification on the quantity and quality of DNA eluates will provide critical information for forensic and ancient DNA researchers to consider before choosing an extraction method. Moreover, the findings of this study should direct efforts toward improving extraction yields and decreasing damage during DNA extraction and purification.

DNA recovered from forensic and ancient DNA sources is generally expected to be in low copy numbers and degraded with regard to strand length. However, considering the following equation, it is important to realize that the exact amount of recoverable DNA from any source is unknown: net yield of DNA=original amount–loss in sampling–loss in extraction/purification–loss due to amplification bias (e.g., due to Polymerase Chain Reaction [PCR] inhibitors).

In most cases, these variables are unknown or poorly documented. This is also true regarding the degree of fragmentation caused by extraction and purification methods. Thus, it would be useful to have some estimation of these variables prior to initiating the study of compromised materials. In this study, the objectives included estimating: (1) the amount of DNA lost during DNA extraction and purification; and (2) with regard to strand length, the degree to which molecules are damaged as a result of the extraction method.

Four DNA standards were created from freshly extracted pig (*Sus scrofa*) liver DNA: (1) standard 1 was ~9,000–12,000bp at 90ng/μl (i.e., representing “intact” genomic DNA); (2) standard 2 was ~9,000–12,000bp at 1ng/μl (i.e., representing the conditions of touch DNA); (3) standard 3 was ~200–400bp at 100ng/μl (probably an unrealistic state, but interesting to test, nevertheless); and (4) standard 4 was ~200–400bp at 0.8ng/μl (i.e., representing forensic or ancient DNA). Ten aliquots of each of these standards were then subjected to 14 commercial extraction methods, many of which are marketed as suitable for forensic and ancient DNA applications (QIAGEN® DNA Investigator, QIAGEN® DNA Micro Kit, MinElute® PCR Purification, QIAGEN® DNeasy® Blood and Tissue Kit, Invitrogen™ Charge Switch, MP Biomedicals™ GeneClean Kit for Ancient DNA [Dehybernation A and B], Promega® DNA IQ, Thermo Fisher PrepFiler™, Bio-Rad™ InstaGene Matrix, TaKaRa NucleoSpin® gDNA Cleanup XP, NucleoSpin® DNA Trace, and TaKaRa NucleoSpin® Trace 8). These standards were also subjected to seven published ancient DNA extraction methods. “DNA in” of the standards was compared to “DNA out” using an Agilent® Fragment Analyzer that is capable of simultaneously measuring DNA quantity (i.e., concentration) and quality (i.e., DNA strand length).

Data regarding both DNA loss and fragmentation were obtained. Observations were made of both DNA loss and fragmentation. Some kits performed very well, whereas others were associated with tremendous loss and/or fragmentation. This indicates that DNA recovered from compromised sources may, in fact, be more concentrated and intact than is generally recognized. It also demonstrates that many extraction methods used to study degraded DNA are far from optimal, and that research should be focused on improving their efficacies.

#### Low Copy Number, Standards, Fragment Analyzer



### B76 Where, Oh Where, Has the DNA Gone?

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**Learning Overview:** The goal of this presentation is to provide attendees with a better understanding of the potential for DNA loss from degraded and low concentration sources when utilizing commonly used silica-based extraction methods.

**Impact on the Forensic Science Community:** Findings of this study will impact the forensic science community in the development of more efficient DNA extraction protocols that specifically retain DNA from degraded and low copy number sources.

The behavior of aged, degraded, and/or low copy DNA during routine laboratory methods is still poorly understood. Previous research on this topic is sparse, but has demonstrated that ample amounts of DNA are inadvertently lost during extraction and purification. These observations suggest that there may actually be more DNA in compromised sources than is commonly recognized. Notably, some DNA extraction and purification methods cause DNA eluates to become lower in copy number than expected, or than would be useful for downstream applications. Considering the case of silica-based extractions, DNA can be lost via two mechanisms. First, it is possible that not all the source DNA binds to the silica column (i.e., DNA could be found in the flow through). Second, it is possible that DNA is not efficiently eluted off of the silica (i.e., DNA could be retained on the silica column).

In this study, DNA standards of known concentration were subjected to the QIAGEN® DNeasy® Blood and Tissue Kit (a silica-based extraction) to identify: (1) how much DNA has not bound to the silica, and (2) how much DNA has not been eluted off the silica column. In order to do so, DNA standards were created using genomic porcine DNA obtained from Zyagen. “Intact DNA” was represented by Standard 1Z, with a peak fragment length of 20k bp, at a concentration of ~100ng/μl. To represent “degraded DNA,” standard 3Z was sheared by sonication to a peak fragment length of roughly 300bp. Low copy variants of each of these standards were created through dilution of standards to <1ng/μl (Standard 2Z and 4Z).

High concentration standards 1Z and 3Z were extracted using the DNeasy® kit. After following the provided extraction protocol, each spin column was moved into a new collection tube and another 200μl of buffer AE was used to elute the DNA that potentially remained in the column. For Standards 1Z and 3Z, this was repeated until the column was eluted a total of ten times in ten separate collection tubes.

To determine if DNA fails to bind onto the columns, the flow through from the purification steps of the initial extraction was passed across a new spin column, washed, and eluted with AE buffer. DNA remaining on this column was eluted into new collection tubes a total of ten times. This was repeated eight more times, resulting in a total of total of 100 eluates from the original DNA. Low copy standards 2Z and 4Z were similarly tested, but silica columns were eluted five times, and four new silica columns were used to attempt to capture DNA from the four flow throughs (totaling 25 tubes). The DNA that was contained in these tubes was quantified using a Qubit® Fluorometer three times. The eluates were run on a Agilent® Fragment Analyzer to determine the size of the DNA fragments that were recovered in each step.

Molecules from all standards were recovered during this process, but detectable amounts varied by method used. For example, standard 1Z eluates had detectable amounts of DNA in 40 eluates (Qubit®) versus 56 eluates using the Fragment Analyzer. However, some general trends were detected. First, molecules were detected in the flow through of all standards, especially those at higher concentrations. Second, the larger fragments (over 4,000bp in length) were recovered at a higher frequency in initial elutions in a new spin column. Thirdly, smaller fragment standard (~300bp in length) eluates were not detectable using the Qubit®. In total, this suggests that modifications to the extraction protocol may be needed to recover adequate DNA from low concentration, damaged sources.

#### Silica-Based, DNA Loss, DNA Extraction

### B77 **Battling the Backlog: A Novel Bioanalytical System for the Separation and Collection of Intact Spermatozoa**

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**Learning Overview:** The goal of this presentation is to demonstrate a novel technology that addresses the bottleneck separation of male from female DNA in rape kits for potential application to the forensic analysis of sexual assault evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a novel method utilizing a Capillary Zone Electrophoresis (CZE) system for the separation of intact cells collected from rape kits. The results presented will invite a meaningful dialog on the optimization and potential implementation of this technology that would significantly improve rape kit analysis speed and efficiency.

The national backlog in sexual assault cases is estimated to be between tens of thousands to half a million untested rape kits. The primary challenge crime labs face in analyzing these kits is the separation of purified male DNA from the mixture of primarily female DNA from gynecological swabs.<sup>1</sup> Standard protocols use Differential Extraction (DE), a manual separation technique that can take upwards of 12 hours to complete. The procedure incubates collected samples in detergents of increasing strength with varying time and temperature environments to differentially lyse fragile epithelial cells prior to stronger sperm cells. The final result contains, at best, a primarily male mixture of DNA that requires a trained analyst in Short Tandem Repeat (STR) mixture interpretation for perpetrator identification.<sup>2,3</sup>

This study addresses the bottleneck challenge of DNA separation in rape kit processing through the use of a novel CZE system.<sup>4</sup> CZE is a promising tool to perform the cell separation and has three major advantages over alternative technologies: a small amount of sample is consumed, which allows for replicate analyses of limited available evidence; rapid separation time compared to standard methods; and single cell detection and collection when interfaced with an automated fraction collector developed in-house. An electrokinetic injection of a simulated sexual assault sample is separated at a high voltage across a capillary with an inner diameter of 100 $\mu$ m. The CZE instrument is coupled with an automated fraction collector that deposits samples eluted from the distal end of the capillary into individual wells on a microtiter plate corresponding to a distinct migration time interval.

Quantitative Polymerase Chain Reaction (PCR) amplification of a Y-chromosome sequence is used to confirm the separation and collection of male DNA from sperm cells in a single well of the microtiter plate in fewer than 15 minutes, representing a significant improvement in separation time compared to current methods.<sup>5</sup> Furthermore, the system was designed to integrate seamlessly within the current work flow of a standard crime laboratory to increase its potential for adoption and implementation in battling the rape kit backlog.

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#### Sexual Assault, Electrophoresis, Automation



### **B78 Optimization of Speed-Vacuum Centrifugation as a Method of Sample Concentration for Successful Analysis of Low Template DNA Samples**

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**WITHDRAWN**



### **B79 The Application of Optical Trapping to Obtain Single-Source Short Tandem Repeat (STR) Profiles From Forensically Relevant Body Fluid Mixtures With Modified DNA Analysis Workflow**

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**Learning Overview:** The goal of this presentation is to demonstrate the utility of optical trapping for separation of a diverse assortment of cells, such as spermatozoa and leukocytes, from forensically relevant mixed samples. Optical trapping is a method used to mechanically manipulate microscopic objects, such as cells. The major operating principle of optical trapping is to direct the momentum of light to impact an object of interest. A laser can be tightly focused through a 100x immersion objective into a point roughly 1 $\mu$ m in diameter. This laser will exert a gradient force on an object, drawing it closer toward the focal point until the gradient force is entirely offset by an on-axis scattering force that traps the object in place. This object can then be moved in three dimensions. Cells caught in the trap and moved on the order of 500 $\mu$ m/s will not be damaged.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a method for deconvolution of DNA mixtures at the beginning of the DNA workflow through the use of optical trapping. This method can be applied to suspected mixed samples to separate a small population of target cells that can then be analyzed within the traditional forensic DNA workflow. Unlike currently in-practice techniques, such as differential extraction, this method can separate a variety of mixtures, such as blood/saliva. This method has the potential to save time at the end of the workflow by negating the need for lengthy mixture interpretation or probabilistic genotyping by generating a single-source STR profile.

The traditional DNA workflow used for analysis of samples that have been separated with optical trapping remains unchanged with only minor modifications. DNA is extracted, quantified, and amplified. Since optical trapping necessarily produces low-copy number samples, samples are concentrated down with vacuum centrifugation prior to amplification. This allows the entire extract to be amplified.

Previous research performed at Virginia Commonwealth University (VCU) has demonstrated that optical trapping can be used to capture and separate both spermatozoa and leukocytes from a larger mixed cell population. Spermatozoa were tweezed out from samples that contained a 1:1 mixture of semen to vaginal fluid in groups ranging from 8–55 cells. Quantification revealed total DNA quantity closely correlated with theoretical yield calculated based on the qualitative observation of the number of tweezed cells. Each sample produced a single-source STR profile with groups of 50 spermatozoa generating STR profiles with greater than 90% of expected alleles. This research demonstrated that small populations of cells could be successfully separated and analyzed from a larger sample; thus, further research was undertaken into other cell types.

A modified transfer protocol was developed that demonstrated improved results from the previous work. Twenty-one samples of leukocytes ranging from 5 to 22 cells were tweezed from liquid samples and analyzed as previously described. Full STR profiles were obtained from 8 of the samples, with 2 of these samples containing only 10 cells. Of the remaining 13 samples, 6 samples had greater than 90% of expected alleles.

#### **Optical Trapping, DNA Mixtures, Cell Separation**





### B80 The Development and Automation of a Swab In-DNA Out Platform Using Dynamic Solid Phase Extraction (SPE)

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**Learning Overview:** After attending this presentation, attendees will have been introduced to a novel, automated microfluidic method for DNA extraction and purification from forensic samples based on dynamic SPE. Additionally, a novel microfluidic lysis protocol and a novel fluidic control strategy will be introduced and applied to forensic genetic sample preparation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing a cost-effective, automated microfluidic technique to lessen sample preparation demands placed on forensic laboratories upstream of genetic analysis.

As the amount of DNA evidence collected in criminal investigations increases, demand for forensic genetic testing continues to grow. The influx of samples to forensic laboratories often outpaces their processing capabilities, leading to a backlog of untested samples. Silica-based SPE techniques are widely accepted forensically, but are time and labor intensive. Although SPE can be automated using liquid-handling robots to increase throughput and reduce contamination risk, these instruments are limited to use in a centralized laboratory. A portable, automated, and multiplexed swab in-DNA out microfluidic platform is proposed for the purification of DNA from buccal swabs using silica-based dynamic SPE (dSPE). The device contains four identical domains to allow for multiple extractions simultaneously. The DNA obtained from this device is amenable to direct amplification by Polymerase Chain Reaction (PCR) and subsequent Short Tandem Repeat (STR) profiling, which represents a significant step toward reducing sample preparation demands placed on forensic laboratories.

Previous work demonstrated semi-automated microfluidic dynamic solid phase extraction of PCR-ready DNA from whole blood, replacing pipetting steps with rotationally controlled fluid flow and magnetic mixing.<sup>1</sup> However, some fluidic control steps required user intervention, preventing complete assay automation. In the work presented, a novel laser-based valving strategy supplants the manual valves, introducing the possibility for complete assay automation. A single laser can be used to both open and close microvalves. This capability is essential for the sequential delivery of the wash and elution buffers required for DNA purification. The dSPE protocol initially utilized three washes to remove both contaminants and PCR inhibitors, including a chaotrope used to facilitate DNA-silica binding and Isopropanol (IPA), which is used to remove cellular components loosely adsorbed to the silica surface. To remove the residual IPA, a Tris/EDTA (TE) wash was implemented. However, as this was the same buffer used to elute the purified DNA from the beads, an appreciable amount of DNA was lost during this step. To prevent this, the TE wash was removed and a novel strategy for IPA removal was implemented. A gas-permeable membrane was incorporated onto the back of the elution chamber to allow for IPA evaporation without sample loss. DNA yields following this modified on-disc protocol ( $0.43 \pm 0.01 \text{ ng}/\mu\text{L}$ ) are comparable to a control reaction done in-tube ( $0.41 \pm 0.16 \text{ ng}/\mu\text{L}$ ), but with the added benefit of heightened reproducibility. The generation of full, 18-plex STR profiles following off-disc amplification and electrophoretic separation of amplicons demonstrates successful inhibitor removal and biocompatibility of assay reagents and device materials. Significant progress has been made toward complete automation of a microfluidic protocol for dSPE-based purification of DNA directly from buccal swabs. This includes integration of enzymatic lysis direct from buccal swab cuttings where the required volume of lysate for dSPE can be delivered directly to the purification architecture through device rotation and valving. Samples lysed and purified on the microfluidic device resulted in STR profiles with 100% correlation to control samples that were prepared in parallel via a conventional, in-tube protocol.

Adopting a microfluidic approach decreases the requirements for reagents and consumables relative to conventional SPE approaches and provides enhanced portability not possible with current forensic biorobotic protocols. Automation and multiplexing represent an important step toward a sample in-DNA out format, ultimately lessening the sample preparation demands exerted on forensic laboratories and increasing throughput.

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#### Forensic DNA, Dynamic Solid Phase Extraction, Microfluidics

## B81 Developing Investigative Leads and Potential Evidence in Wildlife Crime: Consideration of Human DNA Recovery From Handled Eggs and Birds of Prey Feathers

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**Learning Overview:** After attending this presentation, attendees will know that human DNA can be recovered from handled eggs and birds of prey feathers, such that human DNA recovery can be considered in relevant wildlife crime investigations. Attendees will also understand that the sampling method choice will impact the DNA quantity recovered, and that additional studies are required to provide the empirical data to fully inform that choice and maximize the potential of DNA evidence obtained from wildlife-specific substrates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how human DNA can be recovered from handled eggs and birds of prey feathers. This presentation will present an initial dataset to inform the choice of DNA sampling method for these substrates, so that human DNA recovery can be implemented in relevant wildlife crime investigations.

In crimes against humans, it is routine to recover “touch DNA” for the identification of potential offenders.<sup>1</sup> To inform this practice, there are more than 200 published studies investigating the best methods for human DNA recovery from substrates relevant to such crimes, such as bullet casings, knife handles, clothing etc. While some of these could also be relevant to wildlife crime, there are particular substrate types that are wildlife-crime specific, such as animal carcasses, snares/traps, eggs, and feathers. However, there are only four published studies on human DNA recovery from carcasses and snares/traps and no published studies on human DNA recovery from eggs and feathers, although fingermark recovery from these substrates has been demonstrated.<sup>2-7</sup> There is a lack of published empirical data to inform the methods to employ for optimal human DNA recovery within wildlife crime contexts.

This proof-of-concept study examined whether human DNA could be recovered from handled eggs and feathers, and if so, which sampling method would maximize the DNA recovered. Chicken eggs were used as representative bird eggs, and common kestrel (*Falco tinnunculus*) contour and tail feathers (provided by Your Animal Kingdom) as representative birds of prey feathers. A single volunteer handled a cleaned egg or feather in one hand for 30 seconds and the substrate was immediately sampled with either a wet and dry cotton swab or a SceneSafe FAST™ mini-tape. Ten substrates were handled with alternating hands per day; ten eggs on days one and two, and five contour and five tail feathers on days three and four ( $n=40$  in total). Half of the substrates were swabbed and half were mini-taped, and the DNA was extracted, quantified, and profiled.

Human DNA was successfully recovered from both handled eggs and feathers, although in varying quantities. No significant differences were observed between DNA quantities recovered from substrates handled by right and left hands, irrespective of sampling method and substrate type (Mann-Whitney U test,  $p > 0.05$  for all comparisons tested). No significant differences were also observed between DNA quantities from eggs handled on different days ( $p > 0.05$ ) and between DNA quantities from the two types of feathers ( $p > 0.05$ ). As such, to compare the effects of sampling method and substrate type, egg data from both hands and days were combined, and feather data from both hands and feather type were combined. Median DNA quantities of 0.62ng (Interquartile Range [IQR]=0.31) and 0.96ng (IQR=1.03) were recovered from swabbed and mini-taped eggs, respectively, and of 0.22ng (IQR=0.25) and 0.35ng (IQR=0.21) from swabbed and mini-taped feathers, respectively. While the average DNA quantity recovered from eggs by mini-tapes was higher than that recovered by swabs, this was not statistically significant ( $U=46.0$ ,  $p=0.796$ ), presumably due to the wide spread of data obtained using mini-tapes. Use of mini-tapes recovered significantly more DNA from feathers than by swabbing ( $U=22.0$ ,  $p=0.035$ ), and swabbing recovered significantly more DNA from eggs than from feathers ( $U=10.0$ ,  $p=0.002$ ).

This study demonstrates that human DNA can be recovered from handled eggs and feathers and provides an initial dataset to help inform practitioners in their sampling method choice. This dataset needs to be expanded with further studies to investigate the impact of the many variables that affect DNA recovery from exhibits, such as time since DNA deposition, environmental factors, packaging methods, etc., to provide the required empirical data to ensure that practitioners are maximizing the evidence available from substrates encountered in wildlife crime.

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### Wildlife Forensics, Touch DNA, DNA Recovery

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### B82 Pyrosequencing-Based DNA Analysis for Species Identification

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**Learning Overview:** After attending this presentation, attendees will be aware of the development of a quick and robust biological assay for species identification using a pyrosequencing technique.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a novel assay to identify species that were previously classified as non-human miscellaneous samples, as well as providing a novel real-time DNA quantitation assay that is vertebrate-specific.

In crime scenes, unknown biological samples are often human in origin, but some of them belong to other animals. Identifying the source of a biological sample in a crime scene, as well as in wildlife forensic cases, can be critical during investigation. Yet, forensic scientists often lack appropriate standardized protocols to categorize a non-human sample. While previous studies have shown the presence of potential genetic markers to differentiate species, there is still a lack of a quick, easy, and distinct method for species identification from tissue or body fluids.<sup>1,2</sup> Law enforcement agencies, police officers, prosecutors, and defense attorneys expect forensic personnel to have the capability to identify miscellaneous body fluids and tissue. However, no system proposed to date achieves this end using fast, easy, and inexpensive protocols.

With the emergence of novel techniques for DNA sequencing, older, less-specific procedures, such as the use of polyclonal/monoclonal anti-sera, can be updated and replaced.<sup>3</sup> In this study, next generation sequencing methods will be used to analyze a short mitochondrial amplicon that is known to be hypervariable between different species of mammals, birds, and fish. This study will utilize pyrosequencing as well as demonstrating the application on a novel massively parallel sequencer. The resultant sequences can be compared to a large database of known references for species identification.

For this purpose, silica-coated magnetic-particle technology was used to extract DNA from buccal swabs and livestock samples, followed by the SYBR<sup>®</sup> green-based quantitation method using in-house designed vertebrate-specific primers. Amplification and sequencing of 12s rRNA was then performed on the PyroMark<sup>®</sup> Q48 Autoprep instrument. Preliminary sequencing data of 30 different species that are commonly found in a household were aligned and compared. Some samples are from mammals, such as *Canis lupus familiaris*, *Felis catus*, and *Oryctolagus cuniculus*; birds, such as *Gallus gallus* and *Psittaciformes*; and fish, such as *Salmo salar* and *Thunnini*. Results show inter-species variability but intra-species concordance, confirmed with curated databases of known sequences. The goal of this study will be to provide law enforcement with a novel method that can be implemented in forensic DNA labs for a routine and user-friendly confirmatory test for species identification.

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#### Species Identification, Pyrosequencing, Miscellaneous Samples



### B83 A Short Interspersed Nuclear Element (SINE) -Based Multiplex Quantitative Real-Time Polymerase Chain Reaction (qPCR) Assay for Human-Dog-Cat Species Identification and DNA Quantification

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**Learning Overview:** After attending this presentation, attendees will understand the need for a robust animal species identification and quantification assay, principles of multiplex qPCR assay development, and the performance of SINE-based qPCR assays.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by drawing attention to the use of animal DNA evidence in forensics, SINEs as potential markers for forensic DNA applications, and an assay model that could be implemented in animal-related casework.

Although a majority of households in the United States own a pet, resulting in the likely transfer of animal DNA during crimes, this evidence oftentimes remains unutilized by traditional crime labs, which primarily focus on the extraction, quantification, and Short Tandem Repeat (STR) genotyping of human DNA.<sup>1</sup> Historically, there have been numerous cases in which animal DNA has been used to link suspects to victims and/or crime scenes, sometimes serving as the only evidence producing such linkages and resulting in convictions.<sup>2</sup> One way to incorporate animal DNA analysis in the typical workflow of traditional crime labs is to simultaneously quantify human and animal DNA in an evidence sample rather than in separate reactions, allowing for the determination of the presence and quantity of animal DNA without additional consumption of DNA evidence. This information can then be used to inform the analyst's decision of whether to proceed with STR genotyping and for which species.

The purpose of this research was to develop a multiplex qPCR assay using primers and dual-labeled probes that target species-specific SINEs for the simultaneous quantification of human, dog, and cat DNA in mixed forensic samples. Existing animal qPCR assays typically target species-specific mitochondrial sequences or low-copy nuclear genes, each of which has its own limitations.<sup>1</sup> Mitochondrial assays are more sensitive, but less accurate and predictive of STR genotyping success, whereas low-copy nuclear assays are more accurate and predictive but less sensitive. As repetitive nuclear elements, SINEs possess the advantages of both without their respective limitations. It was thus hypothesized that a SINE-based multiplex qPCR assay will be more sensitive, quantitatively accurate, and reliable than previously published assays for quantifying animal DNA.

First, previously published primers targeting human-, dog-, and cat-specific SINEs were validated via endpoint PCR and agarose gel electrophoresis for species specificity and multiplex compatibility with commercial DNA standards.<sup>3,4</sup> Preliminary results indicate that each primer set does not display cross-amplification in the other two species' DNA templates. Bands of comparable brightness were observed in duplex reactions, indicating that human-, dog-, and cat-specific SINE-targeting primers are multiplex compatible. These validation studies were conducted on the GeneAmp® PCR System 9600 in 10µL reactions containing 1x AmpliTaq Gold™ 360 Master Mix, 0.2µM human primers, 0.3µM canine primers, 0.4µM feline primers, and 10ng of each species' genomic DNA standard. The instrument cycling conditions were an initial hold of 95°C for 12 minutes, 40 cycles at 95°C for 15 seconds, 68°C for 30 seconds, and 72°C for 15 seconds, followed by a final extension at 72°C for 7 minutes.

Then, new primers and dual-labeled probes were designed for multiplexing using National Center for Biotechnology Information's (NCBI's) Primer Basic Local Alignment Search Tool (BLAST) and Primer Express 3.0. Human-, dog-, and cat-specific amplicons are 140, 101, and 98bp respectively. qPCR reactions were conducted using the Invitrogen® Platinum II Taq Hot-Start DNA Polymerase kit. The instrument's cycling conditions were an initial hold of 94°C for two minutes, followed by 40 cycles at 94°C for 15 seconds, and 60°C for one minute. Ten microliter singleplex reactions were conducted on the ABI® 7500 Real Time PCR system and contained 1X Platinum II PCR Buffer, 0.2mM dNTPs, 0.2µM forward and reverse primers, 0.1µM Taqman probe, and 0.8units of Taq. Early results indicate a PCR efficiency of 98.1%, an R<sup>2</sup> value of 0.98, and a detection limit of 10pg.

PCR conditions are being optimized in singleplex reactions for each primer/probe set to minimize the Ct value and maximize the amplification efficiency and linear dynamic range. A multiplex detection assay is in development and being optimized for balanced amplification of multiple targets with amplification curves comparable to their respective singleplex reactions. Forensic conditions are being simulated by performing each assay on DNA extracted from mixtures of human, dog, and cat blood at various concentrations.

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#### Animal, DNA, Quantification



### **B84 Using Environmental DNA (eDNA) and Vertebrate Metabarcoding to Characterize Biological Communities: A New Method for Conservation Genetics and Wildlife Forensics**

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**Learning Overview:** After attending this presentation, attendees will better understand the impact of vertebrate metabarcoding on wildlife forensics and wildlife management plans for threatened and endangered species, as well as those of conservation concern.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a novel technique for genetic species identification of unknown vertebrate biological samples for both conservation and law enforcement efforts in combating wildlife crimes.

Illegally poached wildlife products are estimated to bring \$20 billion in annual revenues from black market trading and has been established as a source of funding for terrorism. Poaching causes a dramatic drop in biodiversity, which leads to a further decline of the ecosystem, especially if keystone species, such as elephants, are targeted. Historically, surveys for fish and wildlife have required a significant number of person-hours in the field, using methods that can be stressful to the species being observed. However, the analysis of eDNA, left behind by organisms in water, soil, and air, is revolutionizing biologists' ability to rapidly assess biodiversity. Vertebrate metabarcoding represents an advance in high-throughput DNA sequencing that offers the wildlife community a dramatic improvement in the process of species identification, as it requires only one primer set for all samples, and not species-specific primers.

In this study, the application of the 16S rRNA mitochondrial gene for vertebrate eDNA metabarcoding was explored in Arizona's Verde River, home to more than 50 species of fish and wildlife that are endangered, threatened, or of conservation concern. Replicate water samples of 250mL volumes were collected consecutively from two sites along the river, both prior to and during the annual monsoon season to ensure a robust method in the face of increased organic matter during the monsoon season. Samples were vacuum filtered to collect eDNA onto cellulose nitrate filters. DNA was extracted, amplified by polymerase chain reaction, and libraries were prepared for sequencing on an Illumina® MiSeq® FGx Forensic Genomics System. Sequences were compared to the National Center for Biotechnology Information (NCBI) nucleotide database using the Basic Local Alignment Search Tool (BLAST).

Members of every vertebrate taxon were identified from sequences, including fish, reptiles, amphibians, birds, and mammals. All taxa that were detected corresponded to those known to occur in the Verde River riparian corridor, but there were also new discoveries at the species level.

This technique represents a novel method to characterize vertebrate communities from water samples that is more efficient than quantitative Polymerase Chain Reaction (qPCR), as it does not require species-specific primers, and less stressful to species than traditional survey methods, as no capturing of wildlife is required. Greater than 25 species from diverse taxa were identified with a single water sample collected in a few minutes. The use of eDNA can supplement or potentially someday replace more invasive techniques for surveying wildlife. This method can be used in conservation efforts by wildlife managers to track migration patterns, invasive species, and those of conservation concern, as a few examples. Examples of how this technique could also be applied to wildlife forensics will be discussed. This technique provides the wildlife community with a powerful analytical tool that has wide applicability across conservation and forensic disciplines and will increase the efficiency of investigations and surveys, while decreasing overall cost.

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**eDNA, Vertebrate Metabarcoding, Wildlife Conservation and Forensics**



### B85 Human Identity From the Mosquito Midgut Using GlobalFiler™ Express

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**Learning Overview:** After attending this presentation, attendees will better understand the evidentiary potential mosquitoes have in the forensic community. Complete and partial Short Tandem Repeat (STR) profiles generated from mosquitoes that fed on human blood will demonstrate to the DNA analysts that identification of a person can be obtained from the blood meal, even after several hours of ingestion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees on possible methods and materials that can be used to utilize the minute quantities of blood present in the mosquito's midgut. The use of such a unique substrate could greatly impact the forensic science field.

There are currently three main forms of the *Anopheles stephensi* mosquito: typical, intermediate, and mysorensis. Despite the intermediate and mysorensis forms having poor vector status, the typical form is found in many urban areas and is known to spread various diseases such as malaria. While these insects are generally considered as pests and carriers of such disease, the female mosquitoes can be useful in forensic cases as they feed on human blood. Nucleated white blood cells found in the blood stored in the midgut of the mosquitoes can be useful in identifying the donor on which the female mosquito has fed. In the context of a forensic case, the ingested blood could help identify a suspect or link a victim to a crime scene.

Generation of Short Tandem Repeat (STR) DNA profiles by direct amplification is useful for identification of the donor of the blood that was ingested by the mosquito. Commercially available direct amplification kits allow for detection of the donor of a body fluid and bypasses the labor-intensive and time-consuming steps needed for extraction and quantification of DNA.

Previous research in this laboratory using another direct amplification kit indicated that DNA stored within the white blood cells of the mosquito gut starts to degrade after eight hours post-feed, and the DNA is completely degraded 72 hours post-feed. Partial profiles can still be obtained after eight hours of ingestion of blood. In this current study, mosquitoes were fed on warm blood meals and euthanized at zero hours, four hours, and eight hours post-feed. Several mosquitoes were euthanized at each time interval having fed on a single donor. A mixture of blood from two donors was also used in this study, and these mosquitoes were euthanized at the same time intervals.

This project utilized the GlobalFiler™ Express PCR Amplification Kit to perform direct amplification and generation of STR profiles. To assist in the direct amplification process, COPAN® microFLOQ® Direct Collection Devices were used for puncturing the midgut of each mosquito. These swabs contain a nylon-fiber tip with a lysis agent that helps to break open the nucleated white blood cells. Once a minute quantity of blood was collected on the tips, the tips were cut and placed into the Polymerase Chain Reaction (PCR) amplification tubes where they remained immersed in the direct amplification reagents during the thermal cycling process. A blood sample from each donor was used as reference to compare the results of profiles generated from the midguts of the mosquitoes.

This research indicated that DNA profiles obtained from the mosquitoes using GlobalFiler™ Express amplification kit were consistent between and within each donor. In addition, both donors could be identified in the mixture profiles. Full profiles for all sample types were obtained for zero hours, four hours, and eight hours post-feed. Additionally, it is hypothesized that variability between mosquitoes, both in their feeding and digestion patterns, affected the quality of the profiles obtained. In conclusion, DNA contained in a single or mixed human blood meal ingested by mosquitoes can be useful in determining the donors' identity using the GlobalFiler® Express PCR Amplification Kit. This is a robust method to establish the presence of one or more individuals at a location of interest, within a specific time window.

#### GlobalFiler™ Express, Mosquitoes, Direct Amplification



## B86 Signatures of Suicide in the Human Postmortem Microbiome

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**Learning Overview:** After attending this presentation, attendees will better understand the potential utility of the postmortem microbiome as a tool to aid in classifying forensic death investigation related to suicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into if and which postmortem microbial signatures exist for suicides.

While postmortem microbial communities have the potential to be used as biological biomarkers of forensic relevance (e.g., postmortem interval range indicator), limited data exists on the variability of postmortem microbial communities related to manner of death, especially suicides.<sup>1</sup> This presentation will describe the human postmortem microbiome associations of suicides from samples collected during routine death investigation.

According to the Center for Disease Control, in 2017, suicides are the tenth leading cause of death (second for individuals between the ages of 10 and 34 years) and were twice as common as homicides. As suicides are a significant contributor to deaths in the United States, it is important to identify if potential signatures from the human postmortem microbiome can identify suicides versus other manners of death (e.g., natural or accidental), which may eventually serve as another tool for forensic investigators in determining manner of death.

Postmortem microbial communities from the mouth and rectum were collected using sterile swabs from 44 cases (22 suicides and 22 natural or accidental deaths, matched for similar demographics). All samples were gathered by the Wayne County Examiner's Office in Detroit, MI, during routine death investigations in 2014–2015. Postmortem microbial DNA was isolated and quantified following a modified manufacturer protocol using commercially available kits, a fluorometer, and a microchannel-based automated electrophoresis system. Targeted amplicon (16S rRNA V4 gene region) high-throughput sequencing was conducted on individual sample libraries using a 2 x 250-base pair, paired end approach. Resulting postmortem microbial community data was analyzed using an open-source bioinformatic pipeline, and statistical analyses were implemented using open-source software. Machine learning algorithms were used to identify significant associations among the targeted gene amplicon sequences (postmortem microbiome) and manner of death (suicides versus non-suicides).

Cases in this dataset represented an equal number of samples between males and females aged 19 to 79 years, but cases were not balanced between race. Approximately 90.9% ( $N=20$ ) were White, while the remaining 9.1% ( $N=2$ ) were Black. In addition, the majority of the cases (90.9%) had an estimated postmortem interval range of less than 48 hours. Other variables investigated included antemortem health conditions, location of the event, season of death, and weight status. Overall, initial findings indicate that suicides may, in fact, have distinct microbial signatures present in their postmortem microbiome. In comparison to control data, suicides presented similar patterns of microbial diversity. However, machine learning identified several microbial taxa that may be useful as indicators for assisting determination of the manner of death.

The results from this dataset are important to future research as they provide data about postmortem microbial variability during routine death investigations. It is critical that datasets with larger sample sizes and increased geographic locations (state, national, and international levels) be developed to corroborate distinct microbial signatures for suicides. Further, recent findings surveying the postmortem microbiome have demonstrated postmortem microbiomes can be reflections of antemortem health status, such as with heart disease and tuberculosis.<sup>1,2</sup> This leads to the consideration of whether swabbing for microbial DNA should be required in all death investigations, due to the vast amount of information the postmortem microbial communities could provide about the decedent.<sup>3</sup> With further collaborative research, the suicide microbial signatures may assist in solidifying relationships between the postmortem microbiome and antemortem health conditions. This topic of study is still in its infancy but shows promise for future applications of using microbial communities in future clinical and forensic settings.

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2. Lieberman, T.D. et al. Genomic diversity in autopsy samples reveals within-host dissemination of HIV-associated Mycobacterium tuberculosis. *Nat Med* 22, 1470–1474, <https://doi.org/10.1038/nm.4205> (2016).
3. Carter, D.O. et al. *Forensic Microbiology*. (John Wiley & Sons, 2017).

### Forensic Science, Postmortem Microbiome, Death Investigation



### **B87 The Impact of Environmental Exposure and Chemical Contaminants on Microbial Signature Associated With Forensically Relevant Human Biological Samples**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the microbial DNA associated with various human biological samples and how contamination and environmental changes can impact them.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing understanding in how handling, transport, and storage conditions can alter these microbial signatures, specifically in forensic applications.

Studies such as the Human Microbiome Project have shown that human biological samples can be successfully identified based on their distinct microbial signatures. Identifying these microbial markers not only has the benefit of an additional identification method, but may also be used to supplement current methods to distinguish incomprehensive samples, samples with low human DNA content, as well as having the potential to be implemented into next generation sequencing panels for easy implementation into the forensic workflow.<sup>1</sup> Even though the microbial signatures of body fluids are found to be distinct and stable, the experimental samples often do not compare to those found at the crime scene. Here, environmental exposure or contamination through chemicals or mishandling can often cause severe degradation of forensic evidence.

Venous blood, saliva, semen, urine, feces, vaginal fluid, and menstrual blood were collected from female and male volunteers using Institutional Review Board (IRB) -approved collection methods. This study was designed to investigate how environmental or chemical changes influence the microbial signatures in body fluids. To assess these effects, body fluid samples were exposed to elevated temperatures at various exposure times, detergent, bleach, and Ultraviolet (UV) light. DNA was extracted from the treated samples using DNA Investigator kit with the standard Forensic Casework Sample protocol on the QIAcube for blood, semen, saliva, vaginal secretions, and menstrual secretions according to manufacturer's protocol. QIAamp® Power Fecal DNA kit was used to extract DNA from treated fecal samples, and DNA Micro kit was used to extract treated urine samples, according to the manufacturer's protocols. These treated samples were compared to positive controls (untreated samples) to evaluate changes in bacterial DNA concentration, sequencing read variations, as well as identifying how the microbial taxa would vary between treatments.

Immediately after extraction, the V4 region of 16S rDNA was sequenced on MiSeq® FGx sequencing platform following the dual-indexing protocol as described by Kozich et al.<sup>2</sup> Sequences were then analyzed using mothur version 1.39.5, and statistical analysis was performed using R version 3.5.0.<sup>3,4</sup>

At the phylum level, similar patterns of relative abundance of bacterial phyla were seen between treatments. Major variations of these phyla were seen in the bleach-treated samples in all body fluids. At the genus level, major variations in the relative abundance were seen in the UV-treated blood samples and fecal samples treated with extended periods of elevated temperatures. Menstrual blood and vaginal fluid were least impacted by the treatments, while semen and urine showed the most variation when compared to the positive controls.

In conclusion, the microbial signature-based body fluid identification method is robust and reliable with common environmental extremities and contaminants. Findings from this study will help in minimizing errors associated with the accuracy of the microbial signature-based method for body fluid identification.

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2. Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and Schloss, P.D. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. (2013). *Appl. Environ. Microbiol.* doi:10.1128/AEM.01043-13.
3. Schloss, P.D. et al. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–41 (2009).
4. R Core Team. *R: A Language and Environment for Statistical Computing.* (2013).

#### **Body Fluid ID, Microbiome, Compromised Samples**





### **B88 A Multidisciplinary Effort to Identify the Cat Killer of Silicon Valley**

*Jeremiah Garrido, BS\*, Santa Clara County Crime Lab, San Jose, CA 95110-1717; Christina D. Lindquist, MS\*, University of California Davis, Davis, CA 95616; Michelle L. Bell, BA\*, San Jose, CA 95110*

**Learning Overview:** The goals and key educational objectives that will be highlighted in this presentation include: (1) criminalists working side by side with a veterinarian to identify, document, and collect various physical evidence; (2) the detection and forensic Short Tandem Repeat (STR) analysis of human DNA detected on the feline victims by the Santa Clara County Crime Laboratory Forensic Biology Unit; (3) efforts of the Veterinary Genetics Laboratory Forensic Unit at University of California, Davis to help determine how many different cats the suspect may be associated with harming; (4) screening/characterizing numerous reddish-brown stains that appeared to be non-human blood on various items collected from Robert Farmer's vehicle through presumptive blood testing and quantitative Polymerase Chain Reaction (PCR); and (5) legal aspects and challenges of animal cruelty cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how a multidisciplinary team of criminalists (from various disciplines and forensic laboratories), veterinarians, prosecutors, and law enforcement officers can work together to navigate through the analysis of human and non-human physical evidence in animal cruelty cases.

People often regard their pets as "members of the family," so when numerous domestic cats were reported missing, and subsequently found mutilated and deceased during the months of September and October of 2015, residents of Silicon Valley's Cambrian Area were extremely concerned. A cat killer was on the loose and the number of feline victims was increasing. Given that many individuals who commit crimes against animals are likely to go on to commit crimes against humans, residents of this tight-knit community were rightfully concerned that this could be a serious public safety issue. The case erupted and gained significant media attention following the discovery by San Jose Police Department of a deceased cat in the center console of a vehicle owned by a man in the nearby area named Robert Farmer.

A multidisciplinary team consisting of criminalists (from various disciplines and forensic laboratories), veterinarians, prosecutors, and law enforcement each played invaluable roles in the evaluation of the numerous feline victims, as well as the human and non-human physical evidence that was seized and analyzed. The prosecuting attorney assigned to this case navigated through the complex legal intricacies of animal cruelty law (California Penal Code Section 597[a]) and worked closely with members of the community that were impacted.

In this case study presentation, members of the multidisciplinary team will highlight their efforts to evaluate and analyze the feline victims and the physical evidence that was crucial to linking suspect Robert Farmer to many more victims than was originally anticipated. Some of the key factors that will be highlighted include: (1) criminalists working side by side with a veterinarian to identify, document, and collect various physical evidence; (2) detection and forensic STR analysis of human DNA detected on the feline victims by the Santa Clara County Crime Laboratory Forensic Biology Unit; (3) efforts of the Veterinary Genetics Laboratory Forensic Unit at University of California, Davis to help determine how many different cats the suspect may be associated with injuring or killing; (4) screening/characterizing numerous reddish-brown stains that appeared to be non-human blood on various items collected from Robert Farmer's vehicle through presumptive blood testing and quantitative PCR; and (5) legal aspects and challenges of animal cruelty cases.

The team approach taken proved to be a huge success in providing answers to key case questions and resulted in an increase in the number of counts against Farmer and will be relied upon for future cases of animal cruelty in Santa Clara County.

#### **Animal Cruelty, Cat Killer, Serial Cat Killer**



### **B89 “A Gun Too Far”—Reconstruction of a Homicide**

*Alexander Jason, BA\*, ANITE Group, Pinole, CA 94564*

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**Learning Overview:** The goal of this presentation is for attendees to become familiar with techniques of shooting incident reconstruction in a homicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how evidence evaluation, observational experiments, and graphic exhibits can be utilized in a trial. Attendees attending this presentation will understand the procedures used to investigate a shooting incident, the value of witness statements, and how to make determinations on the dynamics and the limitations of gunshot residue evidence. All of these teaching details are used to integrate discrete evidence elements into a significant shooting reconstruction.

A woman was found fatally shot. It was either a homicide or a suicide: did she shoot herself or was she shot by someone? This issue required experimental testing and modeling. The primary physical evidence at the scene was both the presence and the absence of gunshot residue, a shooting distance determination, and blood spatter. This was a case that required an analytical shooting reconstruction to determine what was and was not possible.

The only witness to the fatal shooting was the boyfriend of the victim. Both the victim and the suspect were in a bedroom when the shot was fired. While there were several other people inside the house (all of whom heard the shot), none of them witnessed the incident. A forensic expert retained by the defense concluded that the incident was a suicide and that the evidence in the form of bloodstains on the victim’s outstretched arm supported his case for suicide.

This case went to trial and the full shooting incident reconstruction was presented to inform the jury about the impossibility of the woman using the particular handgun to shoot herself. Anthropometric data produced by a kinesiologist was used to validate the length of the victim’s arms and the limits of the victim’s reach.

One issue was the early crime scene photos that showed the location and the orientation of the revolver used in the incident. The handling of the firearm by the first arriving police officer was somewhat uncommon, but his actions were very helpful in subsequent analysis of the incident and in the understanding of what could and could not have occurred.

One of the principle evidence items used by both prosecution and defense in the criminal trial was the absence of gunshot residue on the suspect and on the blanket that covered the victim. An identical blanket was obtained and used for gunshot residue testing which, when compared with forensic laboratory testing, provided a valid distance determination. An important part of the reconstruction was to understand and communicate the significance of the gunshot residue evidence to the jury. The limitations of the significance of gunshot residue on a suspect’s hands was researched in the forensic literature and in the guidelines and practices of several forensic and law enforcement agencies. Additional evidence was evaluated by use of a human model to simulate the victim’s ability to hold the firearm.

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### **Shooting Reconstruction, Bloodstain, Gunshot Residue**



### **B90 Crowbar Paints in Forensic Investigations With In-Depth Techniques: A Casework Investigation and the Start of a Multi-Technique Database**

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**Learning Overview:** This presentation addresses the application of highly discriminating multi-technique investigations in crowbar paint investigations, demonstrates how information on the background variation can be obtained, and how these results can be used in reports to assist the court.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how detailed information can be extracted from the paints and used in highly discriminating in-depth comparison investigations, how to obtain information on the meaning of any matches found, and interpret and report this information to the courts.

Crowbar paints are often very discernible (e.g., an orange crowbar paint on a white door frame in a burglary). The preconception was that the paint in many situations consists of a single layer. As in most other forensic institutes, the backbone of the Netherlands Forensic Institute (NFI) paint investigation is the visual investigation and comparison of paints using microscopy and Fourier Transform Infrared (FTIR) investigations. A database of FTIR data with 49 orange/red burglary tool paints is presently used for reference purposes and to assist in interpretation. For interpretation, a likelihood approach is used in which the probability of results in the prosecutors' scenario is estimated relative to the results in the defense scenario. A Likelihood Ratio (LR) range approach is used and expressed in verbal terms.

At the NFI in recent years Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS) is also used in paint investigations. Results will be presented for a single series of five attempted burglaries where orange-red paint found at the site of the burglaries is compared to original paint from a crowbar as found with a suspect, and paints found on the crowbar are compared to original paints from the sites of the burglaries to determine potential paint cross-transfer. Results from microscopic, FTIR, and LA-ICP/MS investigations from this casework investigation complex will be presented and discussed.

Conclusions for these five burglaries ranged from appreciably more probable (LR 100–10,000), through more probable (LR 10–100), and slightly more probable (LR 2–10), to approximately equally probable (LR 1–2) that the investigated object had been in contact with the orange-red crowbar under investigation relative to the situation that the investigated object had been in contact with another at-random orange-red crowbar.

For one attempted burglary, the transferred orange-red paint was clearly different from the crowbar under investigation but might have been transferred from another orange-red crowbar. Therefore, for this specific attempted burglary, no indication was found that the investigated object had been in contact with the orange-red crowbar under investigation.

As part of further improvement of the NFI crowbar paint investigation process, paints from a set of 21 orange/red crowbars and 13 paint samples from orange/red crowbars were investigated using the above techniques. In addition, Liquid Chromatography/Mass Spectrometry (LC/MS) was used to obtain information on colorants.

Overall, more than 50 (organic and inorganic) colorants and some colorant/binding-related organic compounds were identified using LC/MS.

Visual, FTIR, LC/MS, and LA-ICP/MS results will be presented for these paints and discussed. Variation within the paint on a single crowbar using these techniques will be addressed. In this way, information on the background variation (variation in these characteristics for visually similar orange and orange-red paints of crowbars, unrelated to the crime being investigated) can be extracted for these characteristics.

Discussion will include how to use the results from this limited set of paints to assist in the interpretation of matches in crowbar paint comparisons to visually similar paint observed (e.g., at a burglary site).

#### **Crowbar Paint, LC/MS, LA-ICP/MS**



### B91 The Refinement and Application of a Kinetic Model to Predict the Evaporation of Gasoline for Fire Debris Analysis

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**Learning Overview:** The goal of this presentation is to demonstrate refinement of a kinetic model to predict the evaporation of gasoline for use in forensic fire debris analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing the theoretical basis, refinement, and application of the model, which is subsequently used to predict chromatograms corresponding to gasoline evaporated to different levels.

One of the major challenges encountered in identifying gasoline in fire debris is the evaporation that occurs due to the high volatility of the liquid. In the heat of fire, the more volatile compounds undergo evaporation, changing the chemical composition of the liquid. The evaporation process can make comparisons of chromatograms of fire debris samples to ignitable liquids in a reference library more challenging. To address this challenge, reference libraries often contain chromatograms of experimentally evaporated ignitable liquids. However, as experimental evaporations can be time consuming, not every liquid in the library will be evaporated, and many liquids may only be evaporated to a few different levels.

The work presented here describes the refinement of a previously developed kinetic model to predict evaporation rate constants of compounds in ignitable liquids as a function of retention index ( $I^T$ ). In this case, retention indices are used as a surrogate for boiling point or vapor pressure, such that the chemical identity of the compounds in the liquid need not be known. The predicted rate constants are used to determine the total fraction remaining ( $F_{Total}$ ) of the liquid, which is related to the evaporation level. The fraction remaining is then plotted as a function of  $I^T$  to predict chromatograms corresponding to a range of evaporation levels. The model was originally developed and validated with petroleum distillates and, for these liquids, strong correlation was observed between experimental and predicted chromatograms corresponding to evaporation levels of 30%–90% evaporated. However, for gasoline, only moderate correlation was observed between experimental and predicted chromatograms, particularly at higher evaporation levels.

In this work, the model was refined to enhance the predictive accuracy, specifically for gasoline. The original model was developed across the  $I^T$  range 800–2,200; however, gasoline contains very volatile compounds that elute at  $I^T < 800$ . The first step in refining the model was to identify these volatile compounds and to experimentally determine corresponding evaporation rate constants. Unevaporated gasoline samples were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS), and volatile compounds eluting before toluene ( $I^T=750$ ) were identified. A total of 14 compounds with  $I^T < 750$  were identified, including methyl butane ( $I^T=478$ ), hexane ( $I^T=600$ ), benzene ( $I^T=640$ ), heptane ( $I^T=700$ ), and methylcyclohexane ( $I^T=710$ ).

For safety reasons, two-component mixtures of the 14 volatile compounds were then prepared and experimentally evaporated to determine evaporation rate constants. Each mixture was evaporated in replicate in a temperature- and humidity-controlled chamber for up to 16 hours. Samples were removed at various time points during the evaporation process and analyzed by GC/MS using standard procedures. The chromatographic abundance of each compound was normalized and plotted versus time to generate first-order decay curves, from which the experimental rate constant was determined. For example, the experimentally determined evaporation rate constants for heptane, toluene, and octane were  $0.298\text{hr}^{-1}$ ,  $0.168\text{hr}^{-1}$ , and  $0.118\text{hr}^{-1}$ , respectively, which compare well with the rate constants predicted using the original model ( $0.307\text{hr}^{-1}$ ,  $0.189\text{hr}^{-1}$ , and  $0.105\text{hr}^{-1}$ , respectively). The experimentally determined rate constants were then plotted as a function of  $I^T$ , and linear regression was performed to refine the model across the  $I^T$  range 500–800.

#### Fire Debris, Kinetic Model, GC/MS



### B92 Photogrammetry Using Visible, Infrared, Hyperspectral, and Thermal Imaging of Crime Scenes

Gerda Edelman, *Netherlands Forensic Institute, The Hague, 2497 GB, NETHERLANDS*; Maurice Aalders\*, *AMC, Amsterdam, North Holland 1105AZ, NETHERLANDS*

**Learning Overview:** The goal of this presentation is to show the possibilities and challenges of the use of photogrammetry for 3D recording of crime scenes with various layers of information, such as thermal and chemical.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a method for revisiting the crime scene in 3D, including the possibility of extracting chemical and thermal information of the objects in the scene.

Photogrammetry is used to obtain virtual 3D models of objects and scenes. The photogrammetry variant Structure-from-Motion (SfM) uses computer vision methods that detect and match features between multiple images to estimate camera locations and orientations and the 3D structure automatically, without the need for previously placed targets. The technique has proven its value in many application areas, especially in geoscience, remote sensing, and archaeology. In forensic casework, the technique is increasingly used to record the crime scene in its original, undisturbed state. Some application areas where this geometrical mapping is especially useful are the investigation of traffic incidents; for example, high resolution documentation of surfaces of human bodies that have been injured and reconstruction of a suspect's height have been accomplished. The technique also provides a complete and metric 3D registration of objects at the crime scene, thereby allowing digital revisitation of the crime scene after its release, in turn enabling additional measurements and inspection of the crime scene from different angular perspectives. Its rapid, remote, and non-destructive manner of operation renders photogrammetry a powerful analytical tool ideally suited to the needs of both forensic science and forensic casework. Furthermore, its low cost, speed, accuracy, and ease-of-use facilitate its application at the crime scene by non-specialists and, consequently, its introduction into standard crime scene investigation protocols.

Recent developments of advanced imaging techniques have led to the exploration of the potential added value of cameras operating beyond the visible light range for forensic applications, in addition to the standard red/Green/Blue (RGB) imaging. For example, Infrared (IR) imaging can be used to detect gunshot residues or latent blood stains on dark surfaces. Furthermore, Edelman et al. showed that by using hyperspectral imaging (a combination of diffuse reflectance spectroscopy and digital imaging), it is possible to detect, identify, and even estimate the time of deposition of blood traces at the crime scene in an entirely non-contact fashion.<sup>1</sup> Similarly, thermal imaging has proven to be of forensic value in trace detection and is currently also being explored as a tool to measure postmortem human body temperatures. Together, these extra layers of information would be greatly beneficial in the reconstruction of crime scenes, revealing traces, invisible to the naked eye, along with their chemical composition and thermal properties.

In this presentation, several scaled 3D models made from stacks of visible, IR, hyperspectral, and thermal images will be shown using the photogrammetric technique SfM and will demonstrate the benefits for crime scene investigations. While some practical challenges remain to be addressed prior to standard application of the technique at real crime scenes, these results establish SfM as a powerful forensic tool and substantiate the added value of such consolidated data sets in forensic investigations.

#### Reference(s):

- <sup>1</sup> Edelman Gerda, and Maurice Aalders. 2015 Development of a Spectral Camera for Estimating the Age of Bloodstains in Casework. *Proceedings of the American Academy of Forensic Sciences, 67<sup>th</sup> Annual Scientific Meeting, Orlando, FL, 2015.*

#### Photogrammetry, Hyperspectral Imaging, Thermal Imaging



### B93 Forensic Gait Analysis: The State of the Science and a Case Study

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**Learning Overview:** After attending this presentation, attendees will understand the current scientific foundations and limitations of utilizing forensic gait analysis to link a suspect to a criminal based on how they walk (their gait).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight and knowledge into the relatively new, emerging field of forensic gait analysis, which has been used in more than 100 criminal cases in the United Kingdom and is now being increasingly used in the United States.

Forensic gait analysis allows investigators to link (or unlink) suspects to criminals by how they walk (their gait)—evidence that in the past has often been overlooked. With increasing frequency, perpetrators are captured on surveillance video and, at times, even with their faces obscured or hidden; their gait may link the image to a suspect.

In the United Kingdom, forensic scientists in conjunction with government officials are finalizing a Forensic Gait Analysis Code of Practice setting forth guidelines on how forensic gait analysis should (and should not) be utilized. The Code defines forensic gait analysis as "... the analysis, comparison, and evaluation of features of gait to assist the investigation of crime."<sup>1</sup>

Discussion will include how gait analysis was used to show that a person charged with murder walked differently than the perpetrator and will include a co-authored paper on the use of gait as evidence with an analysis of criticisms of the science.<sup>2</sup>

Recent research has established the repeatability and reproducibility of forensic gait analysis using a specific methodology in addition to other factors that may affect the accuracy of forensic gait analysis.<sup>3,4</sup> As a result, this presentation will emphasize the limitations of forensic gait analysis and also its strengths by showing examples from a case study in which an armed robber was linked to a suspect.

Precautions and appropriate qualifications of persons who endeavor to become specialists in forensic gait analysis will be reviewed, in addition to discussing steps experts can employ to reduce bias and increase accuracy during the analysis process.

With video surveillance increasing and devices that enable recording of crimes becoming more widespread, the need for forensic gait analysis will likely grow, and, with biometric advances, the possible future of forensic gait analysis—a mix of technology and expert analysis—will be suggested.

#### Reference(s):

1. Forensic Science Regulator. *Forensic gait analysis: draft code of practice*. 2018.
2. Nirenberg M, Vernon W, Birch I. A review of the historical use and criticisms of gait analysis evidence. *Science & Justice*. 2018 Jul 1;58(4):292-8.
3. Birch I, Birch M., Rutler L., et al. The repeatability and reproducibility of the Sheffield Features of Gait Tool. *Science & Justice* (2019).
4. Vernon W., DiMaggio J.A. 2017. Forensic gait analysis. In *Forensic Podiatr* 2<sup>nd</sup> Edition (pp. 153-179). CRC Press, 2017.

#### Forensic Gait Analysis, Gait, Video Comparison



### B94 Interdisciplinary Forensic Investigations—Combining Evidence in Complex Cases

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**Learning Overview:** This presentation addresses the combination of evidence in interdisciplinary casework investigations to improve assistance to the courts in using the combined value of this forensic evidence. Attendees will learn how this can be accomplished and how this functions in practice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the strength of forensic science in the use and reporting of combined evidence to the courts.

Single forensic expertise results are already relevant in police investigations and court cases. However, combining results from various expertise areas often contribute even more than the single expertise results. The question is, of course, how to combine these results.

This presentation will address the combination of results from complex interdisciplinary casework investigations at the Netherlands Forensic Institute (NFI) by certified experienced experts (three certified experts out of a population of 136 NFI experts). These three experts have had additional training to be an expert in combining results from specific expertises (pathology, toxicology, DNA, fibers, glass, Gunshot Residue (GSR), guns, digital evidence, etc.). Two other experts are presently trained for this role.

A combined expertise report is produced in these interdisciplinary casework investigations in which the combination of results from the separate disciplines is evaluated using at least two mutually exclusive scenarios, as agreed on by both the prosecutor and the defense. Often, results from the separate expertises need to be re-evaluated under these scenarios, common to all disciplines. Sometimes additional investigations are instigated. Previously, results from each single expertise have generally already been reported separately at source level using scenarios that are tailored to the strength of the specific discipline.

The interdisciplinary scenarios are mostly at the so-called activity level. Currently, the evaluation and reporting of forensic evidence at the activity level is one of the major challenges for the forensic community. Much has been written already on activity level evaluation for trace evidence, such as DNA, fibers, glass, etc. Bridging the gap between source level and activity level interpretation is still a major challenge, however. It promises to bring a wealth of possibilities for a more formal interdisciplinary approach to the evaluation of forensic evidence. In this presentation, a methodology is proposed for the evaluation of interdisciplinary evidence within the likelihood ratio framework.

Prosecution and defense scenarios are analyzed for forensically verifiable “core elements” (often offense-related activities in the case) for which activity level core propositions are formulated. Evidence schemes are introduced in which two main types of evidence relations are identified: serial and parallel evidence.

The evidential value of a serial evidence chain is analyzed in detail and shown to be at least as strong as the weakest link in the chain. Parallel evidence chains from one or more forensic disciplines evaluated in light of the core propositions may be combined, taking into account possible conditional dependencies.

The likelihood framework in combination with the evidence schemes allows the expert to combine interdisciplinary evidence in a probabilistic but still intuitive manner. Assumptions and choices are made in a transparent way. If conditional dependencies are present, a Bayesian network may be set up to take into account these dependencies.

At the NFI, these interdisciplinary reports are reviewed by a team of experts: both the experts for the original expertise areas from which results are used in the report, another interdisciplinary expert, and one of the NFI principal forensic scientists.

Specific examples will be presented on how this approach is applied in casework investigations. The present approach is the result of NFI’s experience over the past 20 years in combining results in interdisciplinary casework investigations in one form or another.

#### **Interdisciplinary Investigations, Combining Evidence, Activity Level**



### **B95 See the Forest for the Trees: A Non-Targeted Approach to Discerning Exposure to Explosives Using Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry (MALDI-MS) and the Multivariate Statistical Model Random Forest**

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**Learning Overview:** After attending this presentation, attendees will have learned about the field of touch chemistry biometrics, how this can be applied to establishing an individual's exposure to explosive materials, some of the challenges associated with this type of analysis, and how these challenges can be overcome using a multivariate statistical analysis-facilitated approach to determine compounds that are diagnostic of exposure to explosives.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the benefits of performing MS analysis on latent fingerprint evidence and demonstrate an approach to do so. This presentation will also acquaint attendees with using statistical analysis methods to extract pertinent information from acquired data and use it to establish exposure to a substance when diagnostic ions are not immediately apparent in the mass spectra.

**Hypothesis:** MALDI-MS can be used with multivariate statistical analysis to extract information about which masses detected in latent fingerprints are diagnostic of exposure to explosives.

**Synopsis:** Experiments aimed at detecting and imaging of explosive compounds in fingerprints for the purposes of establishing exposure is the subject of this work. This is accomplished through MALDI-MS imaging and the identification of ions that are diagnostic of a particular explosive. For example, for TNT, a prominent ion at nominal  $m/z$  226 is detected, corresponding to the (M-H)<sup>+</sup> ion. However, for some explosives, intuitively obvious diagnostic ions are not as readily apparent. For example, while RDX produces diagnostic ions by Direct Analysis in Real-Time Mass Spectrometry (DART<sup>®</sup>-MS), these ions are not observed by MALDI-MS.<sup>1</sup> This could be due to interference from the matrix required to ionize the sample or the differences in ionization mechanisms between the two techniques. Nevertheless, exposure to RDX can still be revealed using statistical analysis of the mass spectral data. By using the entire body of information collected from an MS experiment, instead of simply selecting one or two diagnostic ions, a discrimination model that enables identification of indicative fragment ions can be created. The work presented here will demonstrate that MALDI-MS analysis of latent fingerprints, combined with multivariate statistics, can be used to confirm an individual's exposure to explosives such as RDX.

**Methods and Results:** Briefly, five volunteers donated fingerprints for MALDI-MS analysis. Control fingerprints were taken prior to exposure, and RDX-laden fingerprints were taken after donors rubbed their forefingers through ~50 $\mu$ L of dilute RDX that had been dried to a residue on a watch glass. The matrix 9-aminoacridine was then applied to the fingerprints, after which they were analyzed by MALDI-MS. A total of 500 spectra were collected from RDX-laden fingerprints. These spectra were used as the basis for training a Random Forest (RF) model using MATLAB<sup>®</sup>. The RF was performed with two classes: (1) RDX-laden fingerprints; and (2) control prints and background. One-third of the data collected were used for validation of the model. Permutation-based importance of predictive variables was used to extract information about which  $m/z$  values were important in enabling accurate classification of samples. This model was used to successfully discriminate RDX-laden prints from others, with an accuracy rate of 100%. Additionally, the masses predicted as important for discrimination include nominal  $m/z$  102 and 129, which are known RDX fragments whose formation has been studied by both tandem mass spectrometry and density functional theory.<sup>2</sup>

**Conclusion:** The chemical information stored within a fingerprint could be of great value to investigators, though it generally remains underutilized. This information can be probed with MS imaging techniques and used to provide insight about donor exposure to chemicals of forensic relevance, such as explosives. In cases where diagnostic ions are not known or apparent, multivariate statistics can be used with the MS data acquired to reveal which ions are indicative of exposure.

#### **Reference(s):**

1. Sisco E., Dake J., Bridge C. Screening for trace explosives by AccuTOF<sup>™</sup>-DART<sup>®</sup>: An in-depth validation study. *Forensic Science International*. 2013;232(1):160-168.
2. Jeilani Y.A., Duncan K.A., Newallo D.S., Thompson, Jr. A.N., Bose N.K. Tandem mass spectrometry and density functional theory of RDX fragmentation pathways: Role of ion-molecule complexes in loss of NO<sub>3</sub> and lack of molecular ion peak. *Rapid Communications in Mass Spectrometry*. 2015;29(9):802-810.

#### **Mass Spectrometry, Statistical Analysis, Fingerprints**





### B96 Smokeless Powder Additive Profiles and Compound-Specific Stable Isotope Signatures for Potential Brand Identification and Sample Discrimination

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**Learning Overview:** After attending this presentation, attendees will better understand the strengths and limitations of Gas Chromatography/Mass Spectrometry (GC/MS) profiling and isotopic analysis for differentiation, comparison, and potential brand identification of commercially available smokeless powders.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the value of utilizing multiple techniques for the characterization and comparison of smokeless powders that can potentially be recovered from inefficient and/or undetonated Improvised Explosive Devices (IEDs).

Small Arms Propellants (SAP) are readily accessible and cost-effective materials that firearms enthusiasts can acquire for the legitimate assembly of ammunition. Unfortunately, the ease of access to and low cost of these materials is advantageous for their utilization in the construction of IEDs. Typically, the SAP charge is loaded into a metal pipe and sealed with screw-fit end caps. Two recent high-profile domestic terrorist attacks using IEDs (Boston Marathon Bombing and New York/New Jersey attempted bombings) demonstrate their continued usage. Thus, there is a need to develop robust metrics for the characterization of propellants that are used as explosives, as well as for comparisons between exemplar and recovered explosive residues.

The goals of the presented research are to investigate GC/MS additive profiles of SAP and compound-specific stable isotope signatures of SAP for potential brand identification and sample discrimination. One hundred sixty one-pound canisters of SAP were purchased from local firearms stores. The samples represent a wide selection of different distributors ( $n=8$ ) and product brands ( $n=160$ ). For GC/MS additive profiling, aliquots of 250mg for each sample were extracted with 5mL of 3:1 methanol:n-butanol, following the method described by Reardon and MacCrehan.<sup>1</sup> This method was chosen because it preferentially extracts the additives, leaving behind the bulk nitrocellulose. Samples were run using an HP6890 series gas chromatograph equipped with an Agilent® 5973 MSD and an RTX-1 column (30m x 0.25mm ID x 0.25 $\mu$ m). The identified additives were primarily nitroglycerin, ethyl centralite, diphenylamine, and dibutyl phthalate. Of the 110 samples analyzed by GC/MS, 83% were double-base and 17% were single-base powders with 80% of the analyzed powders containing diphenylamine, 45% of the powders containing dibutyl phthalate, and 65% of the powders containing ethyl centralite; 73% of the additive profiles analyzed by GC/MS were the same as at least one other profile. It was noted that many samples that gave the same GC/MS additive profiles could be differentiated by powder granule morphology.

Nitrogen and carbon isotope signatures were measured using isotope ratio mass spectrometry. This was accomplished by loading samples into a Costech 4010 element analyzer. The capsule containing the samples was placed into a heated zone containing chromium and cobalt catalysts along with pure oxygen to convert all of the nitrogen ( $N_2$ ) and carbon to nitric acid (NO) and carbon dioxide ( $CO_2$ ). The helium carrier stream carries the  $N_2$ , NO, and  $CO_2$  to a reduced copper furnace where the NO species are reduced to  $N_2$ . The sample stream then proceeds to a GC column where the  $N_2$  and the  $CO_2$  are chromatographically separated. The separate  $N_2$  and  $CO_2$  peaks are then routed to a ConFlo III interface. The open split in the ConFlo III feeds the MAT 252 MS along with reference peaks of pure  $N_2$  and  $CO_2$ . All peaks were integrated and the  $\delta^{15}N$  and the  $\delta^{13}C$  of the samples calculated relative to the reference peaks in the chromatogram.

The SAP sample isotopic compositions were examined to determine if there were recognizable geographic signatures. SAP manufactured in Canada and Belgium cluster in the most negative  $\delta^{15}N$  and the  $\delta^{13}C$  space. The samples from Finland tightly cluster with negative  $\delta^{13}C$  and positive  $\delta^{15}N$ , thus allowing for discrimination of these powders. Samples from Sweden cluster in the center of the data set. Samples from the United States and Australia tend to show the least negative  $\delta^{13}C$  compositions and the positive  $\delta^{15}N$  signatures. This clustering is encouraging but will require a more complete dataset to draw robust conclusions.

The results from this study show there is a need for the fusion of data from different analytical methods for more robust sample differentiation and comparison.

#### Reference(s):

1. Reardon, M.; MacCrehan, W. Developing a quantitative extraction technique for determining the organic additives in smokeless handgun powder. *J Forensic Sci* 2001;46(4):802-807.

#### Smokeless Powder, Improvised Explosive Device, Additive Profiling



### **B97 A Study of the Thermal Decomposition of Nitrate Ester Explosives by Gas Chromatography/Vacuum Ultraviolet Spectroscopy (GC/VUV) and Its Application to Post-Blast Debris**

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**Learning Overview:** After attending this presentation, attendees will be better informed about the thermal decomposition of nitrate ester explosives and its impact on analyzing realistic post-blast debris.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing a recently developed detector for GC/VUV and its applicability to the analysis of nitrate ester explosives and post-blast debris.

The method employed in this research consisted of an Agilent® 7890B GC (50°C hold 0.5min, ramp 20°C/min to 280°C) with a multimode inlet (50°C to 280°C at 900°C/min), a hydrogen carrier gas (3.2ml/min flow rate), and a Restek® Rtx-5MS column (15m x 0.32mmID x 0.25µm) was utilized in line with a VGA-101 VUV spectrometer. The VUV spectrometer was operated with nitrogen as the make-up gas (0.35psi) and a transfer line/flow cell temperature range of 190°C to 300°C between 125nm and 430nm. This method was utilized to analyze the thermal decomposition of four nitrate ester explosives: NG, EGDN, ETN, and PETN, as well as post-blast debris of double-base smokeless powders.

Nitrate ester explosives thermally decompose in the transfer line of the VUV detector into nitric oxide, carbon monoxide, formaldehyde, oxygen, and water. The explosives decompose into differing stoichiometric ratios of the decomposition products and contribute to variation in each nitrate ester explosive VUV spectrum. Due to adherence to Beer Lambert Law, the percentage of each decomposition product was determined by spectral subtraction. The percent contribution of the decomposition products at 300°C was optimized using the Excel® Solver tool. The explosives decreased in concentration with increasing temperature in a logistical fashion. Using JMP software, a 2 parameter logistic fit was utilized to identify the inflection point (the point at which 50% of explosives were degraded) of the sigmoidal curve. These temperatures ranged from 243.74°C to 248.23°C.

The sensitivity of the GC/VUV for nitrate ester explosives was evaluated by looking at the peak area over the range of transfer line/flow cell temperatures. Results indicated that at lower temperatures, NG and ETN had greater sensitivity, while PETN had variable responses and EGDN did not have significant changes in peak area with changing temperatures. Limits of detection for these compounds analyzed with a transfer line/flow cell temperature of 240°C were calculated at low ppm.

To investigate the applicability to forensic post-blast debris analysis, the Indiana State Police Bomb Squad assisted in obtaining realistic samples of post-blast residues of double-base smokeless powder (Alliant Red Dot) on galvanized steel and Polyvinyl Chloride (PVC) pipe fragments. The pipes were placed in a perforated steel box prior to initiation to allow for collection of the post-blast debris for analysis. These samples were analyzed using the same method described above at a transfer line/flow cell temperature of 240°C. The analysis of the post-blast debris of these two types of devices showed that, for the first time, GC/VUV can be used to identify NG as the energetic material with diphenylamine and ethyl centralite as additives in post-blast debris.

#### **Gas Chromatography, Vacuum Ultraviolet Spectroscopy, Explosive Analysis**



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### **B98 An Update on the Academy Standards Board (ASB) Firearms and Tool Marks (FATM ) Consensus Body**

*Gregory E. Laskowski, MPA\*, Criminalistics Services International, LLC, Bakersfield, CA 93308*

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**Learning Overview:** The goals of this presentation are to describe to attendees the purpose of the ASB FATM Consensus Body and to present updates as to the Body's work on developing and approving standards in the disciplines of firearms and tool marks analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees information on what the mission of the FATM Consensus Body is and the progress it has made and is making in creating standards in the area of firearms and tool mark examinations.

The American Academy of Forensics Sciences created the Standards Board (ASB) as a response to the need for standardization from the forensic community. This was in line with recommendations in the 2009 NAS Report.<sup>1</sup> It is now working in cooperation with the Forensics Science Board of the Organization of Scientific Area Committees (OSAC). The purpose of the FATM Consensus Body is that it focuses on standards and guidelines related to the examination of firearm and tool mark evidence. This includes the comparison of microscopic tool marks on bullets, cartridge cases, and other ammunition components and may also include firearm function testing, serial number restoration, muzzle-to-object distance determination, and analysis of tools and tool marks.

This presentation will discuss the inception of the ASB, its legal status as an entity, its membership, its mission, a discussion on its consensus bodies with particular emphasis on the discipline of firearms and tool marks. Created in 2016, and under ASB Secretariat Brad Wing, the FATM Consensus Body is made up of 33 members representing a number of disciplines that include subject matter experts, general interest, consumer groups, user/government (federal, state, and local), academia, producers, and user/industry. Besides members, there are observers, as well as managers from the ASB. Observers and other non-members are encouraged to participate by reviewing documents and offering comments. Standards can be developed by the Consensus Body or offered by other bodies, such as the OSACs. Once documents containing recommended standards and guidelines are submitted to the Consensus Body, they will be reviewed by members of that body, and changes to those documents may be made to meet American National Standards Institute (ANSI) publishing guidelines. Once the documents have been reviewed, they will be offered up for public comment. Any and all public comments must be reviewed and addressed by the Consensus Body. After final review, the documents will be published as guidelines or standards.

It is imperative that all members participate, and that those individuals and bodies affected by proposed standards and guidelines participate by subscribing to FATM Consensus Body communications, listen in to meetings, which are broadcast on Join Me, read the documents, and offer constructive comments.

#### **Reference(s):**

- <sup>1</sup> National Research Council (NRC), Committee on Identifying the Needs of the Forensic Science Community. (2009). *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press.

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#### **Standards, Firearms, Tool Marks**



### B99 Forensic Implications of a New Polymer Bullet

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the manufacturing process for this non-traditional polymer bullet, its behavior at impact with some common substrates, and its composition and microscopy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the challenges to correlating a polymer bullet to the firearm using comparison microscopy.

A few years ago, a new entry into environmentally friendly ammunition appeared on the commercial market. PolyCase® Inceptor® ammunition, out of Savannah, GA, has created a new bullet design and construction. PolyCase® Inceptor® ammunition has some novel design features in all of their marketed products, specifically the construction and unique shape of their ARX® Defense bullet. Prior studies have shown that traditional jacketed and hollow point bullets have predictable interactions with many yielding and non-yielding intermediate substrates. The newly marketed ARX® Defense bullet behaves quite differently than previous bullet designs. Not only is the bullet lead free, it is comprised of a metal-polymer matrix, manufactured using an injection molding process. This manufacturing process does not lend itself to a traditional hollow point design; however, the bulletsmiths at PolyCase® Inceptor® ammunition have devised a unique alternative to a cavity in the form of three large “flutes” in the ogive of the bullet. The resulting non-expanding design of the ARX® Defense bullet is lightweight and, thus, travels faster than its lead or jacketed counterpart.

To determine the ingredients of this new line of polymer bullets, samples were deconstructed by dissolving the polymer in a suitable solvent, which allowed the metal spheres to settle to the bottom of the vessel. The metal spheres were assessed with scanning electron microscopy/energy dispersive spectroscopy, which as the manufacturer acknowledges, were found to be copper. Infrared spectroscopy revealed that the polymer is nylon. Nylon is by no means a newcomer to ammunition, as it has been used as a coating in some calibers, notably .38 Special, for decades. However, in the old S&W® and Federal® offerings, whether in .38 Special or their other calibers, the nylon merely encased a lead core. This is where the PolyCase® Inceptor® ammunition differs substantially, as the lead core has been eliminated.

Several calibers and styles of PolyCase® Inceptor® ammunition were test-fired at various barriers, including concrete block, gypsum drywall, paneling, plywood, architectural glass, windshield glass, and sheet metal of different thicknesses or gauges. Automobile sheet metal provided the most characteristic bullet holes when perforated by the fluted ARX® Defense bullets as compared to traditional hollow point or full metal jacket bullets. The fluted ARX® Defense bullets consistently created holes in the sheet metal that were triangular in shape. The larger the caliber, the larger the size of the triangular bullet hole. The results were replicated using firearm barrels with both left-hand and right-hand twist directions, and different barrel lengths, which offered slightly different velocities as measured with a chronograph near the muzzle of the firearm. These variables did not have a visible effect on the shape of the holes created.

In addition to assessing the bullet hole itself, high-speed photography revealed that a piece of metal was being dislodged from the substrate by the bullet’s impact. To capture this “punchout” or “plug,” ballistic gelatin was placed near the anticipated exit hole of the bullet, which allowed recovery of many of the small metal pieces that were dislodged. Interestingly, these metal pieces were also triangular in shape, whereas punchouts or plugs from traditional hollow point or full metal jacket bullets are rosette or circular, respectively.

Last, comparison microscopy was attempted in an effort to associate a fired bullet to the firearm from which it was fired.

#### **Polymer Bullet, Inceptor® ARX® Ammunition, Microscopy**



### **B100 The Power of Statistics and Machine Learning Applied to Orthogonal Rapid Methods for the Identification of Inorganic Gunshot Residue (IGSR) and Organic Gunshot Residue (OGSR) Markers**

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**Learning Overview:** After attending this presentation, attendees will be aware of a novel fast approach to detect IGSR and OGSR and machine learning tools to interpret the data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a method to rapidly detect GSR that could provide investigative leads and improve intelligence operations for firearm-related crimes, which could create a greater sense of well-being in society, considering the risk to public safety posed by firearms.

Machine learning is a powerful tool for providing rapid information in our data-driven world. Reliance on these algorithms extends to everyday life in order to search the internet, to provide directions when traveling, to protect devices using facial recognition, and to enhance the reliability of a medical diagnosis, to name a few applications. Machine learning can be applied to the identification and interpretation of forensic evidence, providing fast results when time is of the essence. Firearm-related investigations is a field in forensics that requires fast decision-making. In one example, acoustic systems have been adopted in more than 90 United States cities to detect the location of possible gunshots and allow for police response to the scene in a matter of minutes. In another example, the current method of detection of GSR from the hands of individuals of interest is time-consuming and cannot deliver results fast enough to provide efficient investigative leads. This research presents the development of a rapid method for the detection of both IGSR and OGSR using two orthogonal technologies: Laser-Induced Breakdown Spectroscopy (LIBS) and Electrochemistry (EC), with machine learning for data interpretation.

A study was conducted on 100 background samples and 80 known shooter samples collected using conventional GSR stubs (carbon adhesive on aluminum Scanning Electron Microscopy [SEM] pin mounts) and processed using two sequential rapid methods on the same sample. First, LIBS was conducted using micro-chemical mapping to provide IGSR detection within less than two minutes per sample. Following LIBS, EC was performed on the same stub. The ablation area was spiked with 50 $\mu$ L of acetate buffer pH 4.5 followed by 50 $\mu$ L of acetonitrile, which was evaporated and reconstituted with the aqueous fraction before being applied to a disposable Screen-Printed Carbon Electrode (SPCE). Square-wave voltammetry was applied for an analysis time within five to ten minutes. Lastly, data preprocessing was performed for the application of three different prediction algorithms: Naïve Bayes, logistic regression, and a neural network.

LIBS allowed the detection of IGSR markers (lead [Pb], barium [Ba], antimony [Sb], copper [Cu], aluminum [Al], zinc [Zn]) while electrochemistry complemented the detection of IGSR (e.g., Sb, Cu, Zn, and superior detection of Pb) and also identified the presence of OGSR compounds, such as 2,4-Dinitrotoluene (DNT), Diphenylamine (DPA), Nitroglycerin (NG), Methyl Centralite (MC), and Ethyl Centralite (EC). The performance of these methods was evaluated individually, then combined. Alone, the accuracy of the LIBS method was better than 86%, and the EC method was better than 75%, depending on the classification method. When the two techniques were combined, the accuracy improved to better than 92%.

In the future, this study will be expanded to include modern ammunition that could lack the typical GSR markers of Pb, Ba, and Sb to test the ability of machine learning to differentiate between different classes of ammunition. In addition, samples from individuals with professions or hobbies where false positives are common will be included and deemed a “high-risk” sample population. The high accuracy of the combined methods fills a critical need in the forensic science community for a rapid technique for the detection of GSR.

#### **Gunshot Residue, Machine Learning, Rapid Technology**



### **B101 The Characterization and Detection of Organic and Inorganic Firearm Discharge Residue (FDR) Using High-Performance Liquid Chromatography-Triple Quadrupole (HPLC-QQQ) and Host-Guest Chemistry**

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**Learning Overview:** After attending this presentation, attendees will better understand a novel method developed for the detection of Inorganic Gunshot Residues (IGSR) and Organic Gunshot Residues (OGSR) by LC-QQQ and how its use in forensic laboratories could enhance interpretation of GSR evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing toward the enhancement of the evidentiary value of FDR by providing dual detection of IGSR and OGSR, as well as assisting in the understanding of metal-ligand interactions for other types of applications (e.g., environmental extractions, drug delivery, and organometallics).

FDR or GSR contains both organic compounds (OGSR) and inorganic particulates (IGSR) originating from the powder and primer, respectively. When fired, the heat of the ignition burns the powder and propels a bullet or pellets forward. After the projectile leaves the barrel, a plume of burned and unburnt particulates and powder are spread into the surrounding environment and attach to nearby persons and objects. The current standard practice for GSR analysis relies on the identification of particle morphology and elemental composition of IGSR (American Society for Testing and Materials [ASTM] E1588-17). Nonetheless, there is still a need to complement the examination of IGSR by detecting organic residues' markers. Advances in multiple analytical techniques allow for the detection of OGSR in the ppm and ppb range and IGSR in the ppb to ppt range using Liquid Chromatography/Mass Spectrometry (LC/MS), Gas Chromatography/Mass Spectrometry (GC/MS), and Inductively Coupled Plasma/Mass Spectrometry (ICP/MS). The goal of this project was to perform a simplified confirmatory analysis of both IGSR and OGSR components using the same sample, the same column setup, on one instrument, via an HPLC-QQQ.

In this work, 11 analytes were under investigation: barium (Ba), lead (Pb), antimony (Sb), zinc (Zn), copper (Cu), N-nitrosodiphenylamine (N-NDPA), 4-nitrodiphenylamine (4-NDPA), 2-Nitrodiphenylamine (2-NDPA), Diphenylamine (DPA), Ethyl Centralite (EC), and Methyl Centralite (MC). To be analyzed by QQQ, the inorganic constituents must interact with larger, transporter molecules to traverse the columns toward the detector, whereas the organic molecules require no modification for characterization. The proposed idea includes two different types of binding agents, a cavitand (crown ether species) and a chelating agent (tartaric acid). A pre-column/column setup was used, consisting of a Polymeric Reversed-Phase (PRP) guard column with a trimethylammonium functional group for the tartaric acid and a Pentafluorophenyl (PFP) column for the separation and retention of crown ether complexes and organic constituents. Multiple Reaction Monitoring (MRM) methodology of HPLC-QQQ analytes was used for confirmation of isotope ratios and patterns for these analytes.

Furthermore, to corroborate the accuracy of the technique in characterizing and identifying Metal-Ligand (M-L) complexes, molecular modeling and High-Resolution Mass Spectrometry (HRMS) techniques such as Orbitrap™ were implemented. This work will demonstrate the extraction of multiple species from a single hand swab and address the efficiency of crown ether and tartaric acid complexation with the metals and species formed after the firing event. The validation of the proposed method includes analysis of standard solutions and a set of 50 blind samples collected from the hands of individuals who fired a gun and from those who had not handled a gun. Selectivity, sensitivity, and error rates are reported for the blind set. Limits of detection in the order of 1ppb to 10ppb and limits of quantitation between 3ppb to 30ppb were observed for all analytes of interest, with intra-day and inter-day precision better than 5% Relative Standard Deviation (RSD).

#### **Gunshot Residue (GSR), Simultaneous Detection Technique, HPLC-QQQ**



### **B102 A Study on the Transfer of Gunshot Primer Residue (GSR) From Fabric to Other Surfaces**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the potential for GSR particles to transfer from fabric surfaces onto other surfaces.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing practitioners insight into the dynamics of GSR particle transfers from clothing to other surfaces. This presentation also describes the methods used to test this theory of transfer of GSR.

GSR is produced by a firearm when it is discharged. The primer for centerfire cartridges is mainly composed of lead styphnate, barium nitrate, and antimony sulfide. The residue from the primer explosion escapes from openings in the gun and is deposited on nearby surfaces, including a person's hands and clothing. These particles can be collected and analyzed using automated Scanning Electron Microscopy/Energy Dispersive X-ray Spectrometry (SEM/EDS). Characteristic GSR primer particles have a molten appearance and are composed of barium, antimony, and lead.

There have been numerous studies addressing the potential for the transfer of gunshot primer residue from hands to other surfaces (hands to hands, hands to clothing, etc.) However, there is a lack of research addressing the potential of GSR transfer from fabric to another surface. In this study, three types of transfers are addressed: transfer from one piece of cloth to a second clean piece of cloth, transfer from a piece of cloth to clean hands, and tertiary (and more) transfers between fabric surfaces.

Oftentimes, investigators will submit multiple items of clothing in the same paper bag and ask for GSR analysis on all the items. Occasionally, questions arise of not only whether GSR was present, but specifically where on the garments the GSR particles were recovered from. In order to answer this question, the GSR analyst needs to be able to assess the potential for GSR transfer between two pieces of fabric. This portion of the study consisted of shooting a cloth target, then placing a clean target into contact with the shot target.

In some shooting cases, a person of interest will state that they did in fact touch the victim either to perform Cardiopulmonary Resuscitation (CPR) or check to see if the person was alive. Since more gunshot primer residue escapes from the barrel than from near the handle, the majority of both homicide and suicide victims have gunshot primer residue on their person. To answer the question of whether or not this is a reasonable explanation for the presence of GSR on the hands, a study was conducted in which a target was shot at and an individual with clean hands touched the target near the bullet hole to determine if GSR can transfer from a comparatively porous surface (the cloth target) onto a comparatively smooth surface (the hands).

There have been several studies that examine the transfer of gunshot primer residue to the interior of police cars from a person who has gunshot residue on their person. There have not been studies conducted that try to determine the number of times gunshot primer residue particles could transfer from one surface to another. An analyst who does not handle firearms either in the lab or at home spent a day of training in the firearms section. At the end of the training, the analyst was asked to stub her clothing, her cubicle chair, the driver seat of her car, and any chairs that she may have sat in at her home in the same clothing she had worn to the training. These stubs were analyzed using SEM/EDS instrumentation using standard laboratory procedures for the analysis of gunshot primer residue.

This presentation will detail the results of a series of studies on the transfer of gunshot primer residue from clothing to clean areas that come in contact with the GSR-contaminated clothing. This research takes a novel approach by investigating the likelihood of transfers from clothing to other surfaces, including hands.

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#### **GSR, SEM/EDS, Locard's Theory**



### B103 The Relationship Between Terminal Velocity and Glass Fracture via .177 Caliber Steel BBs

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**Learning Overview:** After attending this presentation, attendees will have gained knowledge in the study of low-velocity projectile impacts and the resulting fracture patterns on double-strength architectural glass. Attention will be focused on the Doppler radar system and its measurement of projectile velocity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the effects of low-energy projectiles on architectural glass, which can be helpful in reconstructing glass fracture patterns at crime scenes.

Glass is an abundant material in the environment and can also be an abundant material found at crime scenes. It may often be the only thing between a bullet and its intended target, which is where fractography becomes necessary. “Fractography is the means and methods for characterization of fractured specimens or components.”<sup>1</sup> The majority of bullets will perforate or shatter most categories of glass; however, in some cases, the bullet may not have sufficient energy to shatter the glass at all. Correlating terminal velocity to the degree of glass fracture can provide useful information regarding the muzzle-to-target distance for a particular firearm/ammunition combination.

Mechanical glass fracture from a projectile impact can result in different types of fracture patterns, including radial and concentric fractures. The degree of fracture depends on several factors, including: type of glass, the thickness of the glass, curvature (if any), distance from the muzzle, and type of projectile. This research focuses on the terminal projectile velocity of .177 steel BBs shot from a pneumatic air rifle. Double-strength glass panes, of 1/8" thickness, were chosen due to its common use in commercial and residential properties. A Doppler radar system was used to measure the projectile velocity from muzzle to target and potentially beyond the target for perforated targets.

Other research has been presented on the subject of dynamic impact glass fracture using timekeeping instruments such as traditional chronographs.<sup>2-5</sup> The use of a Doppler radar system has the advantage of coupling Doppler processing with pulse radars to provide accurate velocity information with superior precision.

Pilot studies have been conducted to ascertain suitable muzzle-to-target distance ranges (12 to 20 feet), the number of pumps required to pressurize the air rifle propulsion system (one to three), and the means of measuring the ricochet distance for projectiles that do not perforate the glass substrate. The experimental methodology was developed from the pilot study results.

The goal of this research is to characterize the relationship between impact velocity and glass fracture pattern. The results of this study can be used to develop future experiments using other types of firearms and ammunition to study glass fracture patterns. It is intended that this research will aid in the advancement of glass fracture analysis, which can be useful in crime scene reconstruction.

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#### Glass Fractography, Doppler Radar, Terminal Velocity



### **B104 On the Migration Route: Challenges in the Forensic Application of Massively Parallel Sequencing (MPS) in Human Body Identification**

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**Learning Overview:** After attending this presentation, attendees will have an overview of the data obtained using MPS in the personal identification of migrants who perished on their way to Europe.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insights into the challenges of forensic identification as consequences of the migrant crisis in Europe.

The recovery of DNA profiles from unidentified individuals is an essential part of the identification process. The limits of traditional DNA typing, which is based on matching the DNA profile of the deceased to the family references or antemortem samples, is evident in cases without any available sample for comparison. Emerging technologies such as MPS generate more data from the DNA sample, without the need for a reference sample for comparative analysis. Moreover, the assessment of externally visible characteristics and biogeographical ancestry may be of assistance in the police investigation. The latter is additionally relevant in cases of missing persons and unidentified migrants who die when crossing borders. The Western Balkan Route has been one of the predominate migration pathways from Africa and the Middle East into Europe. Croatia is the most southeastern European Union member state on the abovementioned migration route. Recently, the Forensic Biology Laboratory (FBL) of Forensic Science Center “Ivan Vučetić” has received an increased number of human identification cases in which remains were recovered from the river borders between Croatia and neighboring countries. The aim of this study is to assess the applicability of the MPS method in the forensic identification of human remains without any family references and antemortem data.

Blood samples were obtained from well-preserved bodies and teeth and tissue samples from decomposed bodies, respectively. DNA typing was performed using the Applied Biosystems® GlobalFiler® and Y Filer® Plus PCR Amplification kits on AB3500 Capillary Electrophoresis (CE) instrument. Laboratory procedures and data interpretation using Gene Mapper® ID-X v.1.4 were completed according to the accredited workflow at the FBL.

In addition, a library was prepared using the Verogen ForenSeq™ DNA Signature Prep Kit B. A positive 2800M control, negative amplification control, and reagent blank were included. The library pool was sequenced on the Illumina® MiSeq® FGx Instrument. All steps were performed following the manufacturers’ instructions. Subsequent data analysis was performed using ForenSeq™ Universal Analysis Software (UAS) v.1.3 with default threshold settings. In order to estimate biogeographic ancestry and phenotype, the ForenSeq™ UAS and Snipper app v.2.5 were compared. In addition, paternal inheritance was evaluated using Whit Athey’s algorithm using a Bayesian approach.

The run quality metrics (cluster density, clusters passing filters, phasing, and prephasing) were within the recommended range. In total, 231 Short Tandem Repeat (STR) and Single Nucleotide Polymorphism (SNP) loci were typed for each sample, without contamination observed during the DNA typing process. Autosomal and Y-chromosomal Short Tandem Repeat (Y-STR) data obtained using MPS showed concordance with CE data. ForenSeq™ UAS classified four out of five samples in admixed American populations and one sample had European ancestry. However, Snipper assigned one sample in the European cluster, two samples between Europe and South Asia, one sample in the South Asia, and one sample slightly displaced toward the African cluster. This was consistent with the prevalence of determined Y-chromosome lineages. The UAS and Snipper prediction models agreed on the prediction of hair and eyes color. Moreover, the hair color of each individual was concordant with the data obtained from the medicolegal reports while eye colors could not be verified due to body decomposition.

The application of MPS can provide valuable data for personal identification in challenging forensic samples, such as human remains of migrants who die when crossing borders. The prediction of hair color, autosomal STR, and Y-STR data was consistent. However, the discordance was evident in ancestry prediction due to the lack of certain Eurasian population databases such as the Middle East. In the meanwhile, an individual of European ancestry has been identified and, for four remaining individuals, the analysis conducted here may contribute to the ongoing investigation.

#### **Massively Parallel Sequencing, Human Identification, Migration**



## B105 Advances in Microhaplotypes (Microhaps) as a Comprehensive Forensic Marker

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**Learning Overview:** After attending this presentation, attendees will understand the current status of the research conducted on microhaps for forensics applications.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining how microhaps are a comprehensive forensic DNA tool and how their implementation can complement conventional Short Tandem Repeat (STR) -based analysis.

Microhaps are biomarkers less than 300 nucleotides long that display multiple allelic combinations.<sup>1</sup> The main advantages of microhaps over conventional STRs include the absence of stutter, same-size alleles within each locus, lower mutation rate, and ancestry informative alleles. These forensically relevant loci can yield a power of discrimination similar to STRs while enhancing Human Identification (HID), mixture deconvolution, and biogeographic ancestry prediction. Sanger sequencing does not allow determining the cis/trans relationship among closely related SNPs while Massively Parallel Sequencing (MPS) allows determining the parental haplotypes at each locus by clonally sequencing of each DNA molecule if they are included in the same amplicon. Currently, STR panels are used for mixture deconvolution and SNP assays are used for ancestry inference. Microhaps can be used for both functions allowing, for example, to infer the ancestry of a minor contributor to a mixture (DNA intelligence). In this study, the current status of the research conducted in the George Washington-Forensic Molecular Lab (GW-FMB) lab and elsewhere on this new multi-function DNA marker and its potential impact to the field of forensic genetics will be discussed.<sup>2</sup>

A newly developed MPS assay of 74 microhap loci was evaluated on the Thermo Fisher Scientific™ Ion Torrent S5™ system to address different forensic research questions including human identification, mixture deconvolution, and ancestry inference.<sup>3-5</sup> For mixture deconvolution, two- to five-person mixtures at different DNA input were simulated, with each donor with a distinct ancestry and contribution ratio to simulate casework-like DNA samples. Mixture results were compared to conventional capillary electrophoresis-based STR and sequence-based STR analysis. To assist in the interpretation of microhap mixed profiles, two Probabilistic Genotyping (PG) software, LRMixStudio v2.1.4 (semicontinuous) and DNANView MixtureSolutions v18-6-20 (continuous), were adapted to microhap mixture data intake to evaluate the output compared to STRs.

The MPS assay improved the deconvolution of all tested mixtures and complemented results of size-based and sequence-based STR analysis. Overall, PG software facilitated and reduced the analysis time of microhap mixed profiles, proving the amenability of microhap data to casework implementation. These results also point toward the rapid development of new mixture deconvolution tools for the implementation of microhap profiling in casework in the near future as new and extremely polymorphic markers are being discovered. Rare variants have been identified that increase the power of discrimination of the assay and the ability to correctly infer the number of contributors to a mixture. Furthermore, biogeographic ancestry of the minor DNA contributor detected in a 10:1 and 20:1 two-person mixtures was accurately inferred by considering the unique minor alleles reported.

These results indicate that the emerging microhaps are an effective and comprehensive biomarker tool, which can be used to enhance and broaden forensic DNA investigations.

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### Massively Parallel Sequencing, Microhaplotypes, Forensic Applications



### **B106 Assessing the Reliability of Single Nucleotide Polymorphism (SNP) Micro-Array Data for Forensic Genealogy**

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**Learning Overview:** After attending this presentation, attendees will understand how the source of tissue, quantity of template DNA, and degree of degradation affects high-density SNP micro-array results. Additionally, attendees will learn how these variables affect the ability to identify related individuals through the searching of genealogical databases consisting of high-density SNP genotypes.

**Impact on the Forensic Science Community:** The data presented will provide the forensic science community with basic information regarding the effect of DNA quality and quantity on the accuracy and call rate of high-density SNP genotype profiles. The data presented can be used to determine whether DNA extracted from forensic samples will be suitable for SNP micro-array processing used in forensic genealogical applications.

Forensic Genealogy (FG) is a powerful investigative technique to aid in the identification of victims and suspects of unsolved crimes. By establishing the capabilities and limitations of FG, the community will be able to address the inevitable court challenges and more effectively use this technique to provide investigative leads associated with unsolved murders, sexual assaults, and missing person cases.

FG is a three-step process. Step 1 is the extraction and quantitation of DNA from forensic evidence or human remains. Step 2 is the generation of SNP genotypes using either high-density SNP micro-arrays or whole genome sequencing. These steps serve as the genetic foundation of subsequent downstream genealogical investigation. The final step is comparing the SNP data to genealogical databases by a professional genealogist.

In this study, the quality of high-density SNP micro-array data using varying amounts of DNA from blood and post-coital samples was evaluated by sending the same samples to three laboratories for either SNP chip or whole genome analysis. DNA extracts (2 to 200ng) purified from blood or post-coital samples were analyzed using the Illumina® Infinium™ National Institute of Standards and Technology Global Screening Array (GSA), the Infinium™ CytoSNP-850K Bead chip, and Whole Genome Sequencing based on the Illumina® NovaSeq™ technology. The SNP micro-array genotypes and sequence results were evaluated on call rates, accuracy (as compared to the whole genome sequence), and reproducibility within and between chips.

DNA extracted from blood and analyzed on the CytoSNP or the GSA chip had SNP call rates that ranged from 88.9% (2ng) to 99.78% (200ng). SNP call rates associated with post-coital DNA and analyzed with the GSA chip ranged from 50% (2ng) to 76% (50ng), whereas the same DNA processed on the CytoSNP array had call rates from 64% (2ng) to 91% (50ng). Intra-chip reproducibility was greater than 99% for samples with >10ng and as low as 97.8% for 2ng samples. Only 104,385 SNPs were common to both chips; concordant SNP genotypes between the two chips at these SNPs ranged from 82% to 89% with blood or post-coital DNA samples that had at least a 70% call rate.

When SNP genotype calls were compared to Whole Genome Sequencing data, the degree of concordance varied based on the amount of DNA, the degree of degradation, and the stringency level of the GENCALL Score using the Illumina® Genome Studio software. All the non-degraded DNA from blood and the post-coital samples with at least 10ng of DNA could be used to reliably match samples entered into genealogical databases, whereas highly degraded DNA with low-template amount could not match samples in the genealogical databases.

#### **Forensic Genealogy, SNP Micro-Array, Degraded DNA**



### **B107 Multi-Marker Match Statistics: Combining Results Across Sequence-Based Short Tandem Repeat (STR) and Identity Single Nucleotide Polymorphism (SNP) Markers**

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**Learning Overview:** After attending this presentation, attendees will understand the considerations for calculating multi-marker statistics in kinship and routine forensic cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by ensuring attendees have an improved understanding of the statistical considerations for combining results across loci/markers, and ForenSeq™ users in attendance will receive actionable guidance.

Analyzing signs of linkage disequilibrium among population samples and father-son pairs will inform decisions regarding statistically combining markers.

Significant progress has been made in the past five years in developing the assays, bioinformatic methods, and population frequency data needed for the implementation of routinely sequencing STR markers. In addition, the forensic community is determining the utility of sequencing identity SNP markers, the smaller targets of which can outperform STRs in degraded samples. When both autosomal STR (auSTR) and autosomal identity SNP genotype data are present for a casework sample and these genotypes are consistent with a person of interest, laboratories are faced with the new challenge of reporting match statistics for a combination of marker types.

At this time, the most likely combination of markers to be genotyped concurrently, due to commercial availability, are the 27 auSTR and 94 Identity Informative SNP loci included in the Verogen ForenSeq™ DNA Signature Prep Kit. In the associated software user guide, the manufacturer indicates that the 121 autosomal genetic identity markers generally meet expectations of independence at the population level but may not be independent for the purposes of kinship analysis.<sup>1</sup> In such cases, they recommend either the use of haplotype frequencies for the pair of loci or performing the calculation using the more informative of the two loci within a particular case.

Tillmar and Phillips discussed the issue of linkage and provided a method for evaluating the effect of linkage in identity and relationship testing.<sup>2</sup> Regarding Linkage Disequilibrium (LD) assessment, this study provides tables of locus pairs within 0.5cM and evaluated these pairs for signs of LD with HapMap data and proxy SNPs as needed (e.g., for all STR loci). For the 121 ForenSeq™ STR and Identity Informative SNP loci, this study reported five pairs located within 0.5cM of one another and none showed signs of LD.

In this study, LD evaluation will be performed between these 121 SNP and STR loci using National Institute of Standards and Technology (NIST) population sequence data for approximately 1,400 samples, including 400 father-son pairs (auSTR data reported).<sup>3</sup> This analysis is representative of the use-case, as it will not require proxy SNPs, and it will be based upon the STR sequences, which are expected to be used for forensic casework calculations. In addition to outlining a method that can be reproduced for other current or future assay/marker combinations, this work will provide refined guidance to ForenSeq™ users on statistically combining results in identity and kinship cases.

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#### **STR, SNP, Linkage Disequilibrium**



### **B108 An Evaluation of the Illumina® Infinium™ Omni Express Exome Bead Chip for Forensic Testing**

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**Learning Overview:** After attending this presentation, attendees will understand the performance of the Infinium™ Omni Express Exome 8 Bead Chip on DNA from blood and bone samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the current benefits and limitations for Single Nucleotide Polymorphism (SNP) chip genotyping of forensic samples.

SNP chips provide hundreds of thousands to millions of loci in a single test. The large number of loci can provide prediction of ancestry, phenotypic information, and relatedness well beyond the limits of Short Tandem Repeat (STR) technology. The main limitation to using Infinium™ SNP chips is that they are designed for use on much higher levels of relatively intact DNA than is found in most forensic samples. Research into the effects of running lower template levels and degraded template on SNP chips is currently lacking. This study aims to evaluate the performance of the ~960,000 SNPs on the Infinium™ Omni Express Exome-8 BeadChip across a range of template amounts from human blood and bone samples.

Blood samples were collected approximately one week postmortem and bones were sampled at four to eight years postmortem. All samples were extracted using QIAGEN® chemistry, showed minimal inhibition and degradation when tested with Quantifiler® Trio, and gave full or nearly full STR profiles when tested with the Globalfiler® system. A DNA dilution series from 250pg to 10ng, for blood and bone samples, was tested using the Omni Express Exome system. The chips were read on an Illumina® iScan® instrument, analyzed in the GenomeStudio® software using the manufacturer recommended settings, and then compared for accuracy, reproducibility, and sensitivity.

The SNP call rates varied by individual tested, were lower for the DNA from bones, and were reduced as the template levels were lowered. The blood and bone DNA at 10ng of template had ~72% and ~30% call rates, respectively. At the lowest call rate, approximately 300,000 SNPs were called. The accuracy of the SNP calls fell dramatically when the call rates were below 70% with up to 50% error rates. Re-analysis in the genome studio software with higher stringency settings lowered error rates but also lowered call rates.

Phenotype and ancestry predictions, using the Paragon® Snapshot® system, for samples with at least a 70% SNP call rate, were consistent with the known hair color, eye color, skin color, and ancestry of the individual. For the samples with less than a 70% SNP call rate, the confidence levels of the predictions were too low to be significant.

The results of this evaluation suggest that the Infinium™ Omni Express Exome SNP chip has potential to be useful for forensic-type sample testing; however, additional development work is needed to increase call rates and accuracy for samples with lower levels of DNA and/or degraded DNA.

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#### **DNA, SNP Chip, Bone**

## B109 DNA Storage Under Multiple Conditions

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**Learning Overview:** After attending this presentation, attendees will understand the long-term storage of DNA under multiple conditions that reflect those in a laboratory setting and those in field work.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing guidelines in regard to DNA storage under multiple conditions that will still maintain functional integrity for downstream forensic analysis.

Extracted DNA stored in a Tris-EDTA (TE) buffer at ambient temperatures of 18–20°C will not show significant degradation in quality or concentration that would impede downstream forensic analysis. Tris-EDTA is a widely used buffer in DNA storage, and it is accepted that DNA can be stored short-term at 4°C and long-term at -20°C.<sup>1</sup> This method of storage is reliant on consistent access to a power source to maintain temperature in the refrigerators and freezers. This is manageable in many first-world countries, but may not be possible in developing countries where fieldwork may be performed due to an increase in sexual violence.<sup>2</sup> It is imperative that DNA be properly maintained in order to be considered legally admissible.<sup>3</sup>

Samples were extracted from a single-source blood donor and spun down in order to pellet the red blood cells. The buffy coat was removed and washed with Deionized (DI) water through repeated centrifugation and removal of the supernatant to concentrate the white blood cells, from which the DNA was extracted using QIAGEN® QiAmp® DNA Investigator.<sup>4</sup> Samples were then eluted into the QIAGEN® ATE storage buffer, DI water, and Invitrogen™ Tris-EDTA. The samples were then pooled by storage media and diluted 1:200, and samples stored at 4°C and -20°C were aliquoted to avoid repeated freeze-thaw cycles. Samples were stored at 4°C, -20°C, 18–20°C, and 35°C in Eppendorf® DNA LoBind tubes and quantified using Quantifiler® Trio every two weeks in order to track degradation trends through the degradation index and concentration.<sup>5</sup> The data obtained from these quantifications will be analyzed using IBM SPSS statistics v23. Once degradation became apparent, samples were amplified using Identifiler® Plus and run through Capillary Electrophoresis (CE) on an ABI® 3500 Genetic Analyzer in order to obtain a genetic profile.<sup>6</sup> As of August 1, 2019, 200 samples have been quantified and amplified, and 80 samples have been run through CE to obtain a genetic profile. Initial attempts to run CE had encountered a technical problem and the samples are being rerun.

The study, as of writing this, has completed 18 weeks of experimentation. DNA stored in DI water at 35°C has shown complete degradation and evaporation at ten weeks with no detected DNA and no detected degradation index. This sample has been discontinued from future quantifications and amplifications.

**Table 1. Degradation index (DI) and concentration (ng/μL) for DNA stored under various conditions at 18 weeks**

	-20°C		4°C		18°C		35°C	
	DI	Conc.	DI	Conc.	DI	Conc.	DI	Conc.
QIAGEN® ATE	0.85	4.7	1.15	4.65	1	3.65	1	4.45
Invitrogen™ TE	0.95	4.2	0.9	0.9	0.8	0.1	0.95	1.05
DI Water	1.1	3.4	0.9	2.5	1.8	0.8	N/A	0

The degradation index is measured between <1 (no degradation/inhibition unlikely), 1–10 (slight to moderate degradation/inhibition possible), and >10 or blank (significant degradation/inhibition possible).<sup>5</sup> The degradation index for all samples at these temperatures have not shown to significantly differ, and the slight levels were not enough to impede downstream analysis. Samples stored under these conditions did not show a significant decrease in concentration that would impede downstream amplification or analysis, or an increase in level of degradation, over the study thus far.

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## DNA Storage, DNA Degradation, Time Study



### B110 Optimizing an Integrated Workflow for Processing Paper Evidence in a Multidiscipline Crime Laboratory

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**Learning Overview:** After attending this presentation, attendees will better understand an optimized multidisciplinary workflow for processing paper evidence for DNA, questioned documents, and latent print analyses. This workflow can be applied to crime laboratories that encounter paper evidence and must share evidence among disciplines.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an evaluation of workflow options in processing paper evidence for DNA analysis while maintaining the integrity of the evidence for other disciplines to process. This presentation will offer suggestions for non-destructive sampling of paper evidence for DNA analysis prior to transfer of evidence to another discipline for further analysis.

Paper evidence is encountered in crimes such as robberies, kidnapping, forged checks, forged prescriptions, and harassment. Traditional methods for processing paper for touch DNA, including cutting pieces of paper and wet/dry swabbing of paper, are destructive to the evidence. Such sampling for DNA analysis may decrease the evidentiary value of the paper for other disciplines (for example, questioned documents and latent prints). Due to the difficulties of processing paper for touch DNA, the flow of the evidence upon submission to the laboratory may start with the questioned documents discipline, then transfer to the latent prints discipline, skipping the DNA discipline altogether. An alternative workflow may involve collecting DNA samples from the paper evidence after it has been processed by the other disciplines. Submitting paper evidence for latent print processing and questioned document analysis prior to DNA analysis may compromise the ability to perform DNA analysis. Both latent print chemical development and questioned document examination may introduce extraneous DNA on the evidence, if clean technique is not used during the processing. As such, the evidence would be rendered unsuitable for DNA analysis after other laboratory sections have completed their testing. An optimized workflow for processing of paper evidence is needed to address the difficulties that may arise in sharing paper evidence among the DNA, questioned documents, and latent print disciplines.

A series of small-scale studies were completed to determine optimal paper sampling for touch DNA, extraction method, latent print chemical development method, and workflow for paper evidence to be shared among the DNA, questioned documents, and latent print disciplines within the laboratory. Evaluation of the data from the small-scale studies and the literature guided the decisions for how the evidence would flow among the three disciplines. A proposed workflow was developed that started with DNA sampling and analysis followed by questioned document examination. Latent print examination was last.

A study of mock evidence using the proposed workflow was conducted by collecting handwritten samples from 12 volunteers. Two paper types, 20lb copy paper and 16lb notebook paper, were tested. DNA samples were collected by dry vacuum swabbing the paper.<sup>1</sup> After swabbing, papers were photographed to document visible markings on the paper, then processed for indented handwriting using the Electrostatic Detection Apparatus (ESDA). After ESDA, papers were returned to their original containers and transferred to the latent print unit for chemical development and evaluation by a latent print examiner. DNA was detected for all samples at quantitation and samples were amplified with the Applied Biosystems® GlobalFiler® PCR Amplification Kit. Profiles were obtained for all samples collected and varied in quality. Profile quality corresponded with quantitation value obtained—the lower the quantitation value, the lower quality of the profile in terms of number of loci with allelic drop-out or peaks below the stochastic threshold. The vacuum swabbing method did not impact the ESDA development of the indented writing for each paper sample. Latent prints of value for comparison were developed for 22 of 24 samples. The results of the testing of the proposed workflow on the mock evidence demonstrate that paper evidence can be shared among DNA, questioned document, and latent print disciplines without loss of evidentiary information. The study gives multidiscipline crime laboratories a framework of how paper evidence should flow through the laboratory once it has been submitted for analysis.

*This research was funded by the Forensic Sciences Foundation Lucas Research Grant.*

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#### Paper Evidence, Touch DNA, Workflow





### **B111 Data Interpretation Guidelines for Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) - Based Forensic Proteomics**

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**Learning Overview:** After attending this presentation, attendees will: (1) gain an appreciation of the unique data interpretation challenges of forensic proteomics; and (2) take home specific guidelines for constructing objective and defensible proteomics assays.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing common practices among the relevant communities and continue to increase visibility and acceptance of this emerging discipline.

Proteomics has become a standard approach to the study of cellular function. Based on LC/MS/MS measurements of fragmented proteins (called peptides) coupled with bioinformatics tools to identify those peptides, proteomics can be used to detect and quantify proteins in a sample. LC/MS/MS instrumentation provides high molecular specificity across a broad range of peptides, allowing for the identification of a virtually unlimited variety of proteins in a single sample. This makes proteomics a desirable alternative to the more traditional single-agent protein assays, such as Enzyme-Linked Immuno-Sorbent Assay (ELISA) and immunoelectrophoresis. As a result, the field of forensic proteomics is a steadily growing area of research. To date, a number of forensic proteomics applications have been explored, including microbial identification, characterization of microbiological growth media, human hair analysis, bodily fluid identification, species identification from bones and other tissues, identification of protein toxins, such as ricin and the botulinum neurotoxin, and the characterization of various historical and archaeological artifacts.

There are two common types of proteomics methods that employ LC/MS/MS: targeted and untargeted. With targeted methods, the instrumentation is tuned to detect a small number of pre-specified peptides. The data produced by targeted LC/MS/MS assays amounts to a list of peak intensities corresponding to the peptides searched for by the instrument. Research and development of targeted assays involves selecting the most protein-specific peptide markers to measure, constructing standard operating protocols, characterizing the limit of detection, and measuring the sensitivity and specificity of the overall method. In this way, targeted proteomics assays are similar to traditional MS-based chemical assays. Untargeted LC/MS/MS methods, while less sensitive than their targeted counterparts, offer the ability to detect a virtually unlimited variety of peptides in a single measurement. They achieve this by operating in data-dependent mode, where the instrumentation is set to detect as many peptides as possible, producing thousands of mass spectra in a single experiment. Because of the vast number and diversity of fragmentation spectra involved, untargeted proteomics methods rely heavily on search databases and bioinformatics tools to identify peptides in an unknown sample and associate those peptides with proteins and/or protein sources. This creates unique data analysis and interpretation challenges that must be addressed when establishing the statistical defensibility of untargeted forensic proteomics methods.

In this presentation, statistical issues associated with the emerging field of targeted and untargeted forensic proteomics will be discussed. Key considerations for meeting the *Daubert* criteria will be presented, including establishing reliability of a method and developing a framework for interpreting the strength of proteomics evidence. The importance of standardized data analysis protocols, objective data interpretation criteria, and appropriate search databases will be demonstrated. An untargeted proteomics method for the detection of protein toxins, such as ricin and abrin, will be presented and used to illustrate the potential of this emerging discipline.

*This work was supported by the Department of Homeland Security Science and Technology Directorate.*

#### **Proteomics, Mass Spectrometry, Protein Identification**



### B112 The Identification of Cadaver Liver Tissues Using Postmortem Transcriptome Biomarkers

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**Learning Overview:** After attending this presentation, attendees will have learned how to use postmortem messenger RNA (mRNA) transcripts in decomposing tissues to gain insight into the identification of tissue segments arising from organs collected from actual cadavers from ongoing criminal cases. Precisely, attendees will understand that RNA is stable in cadavers' liver tissues and is adequate for profiling gene expression and that gene expression signatures of mRNA exposed for up to 37 days of autolysis can be used to identify putative tissue fragments.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the determination of internal organ sources from criminal casework that may assist in death investigations.

The human transcriptome is the complete expression of mRNA transcripts produced in all tissues. Approximately 10% of mRNA transcripts are encoded by tissue-enriched genes and a variety of genes are enhanced to a greater extent in one tissue type, some up to five times the maximum levels of all other tissues. RNA quality is contingent on tissue source; for example, liver and spleen are more abundant in ubiquitous RNases that degrade RNA molecules more rapidly and with a higher activity than less RNase-rich tissues (e.g., heart and muscle).

This study hypothesized that as a human body decays, mRNA profiles will permit organ identification using biomarkers on bona fide autopsy-derived sources. This study is the first of its kind to use actual cadaver tissue from ongoing criminal cases in a postmortem transcriptome identification assay. To test this hypothesis, total RNA was extracted and the approach was prioritized by performing targeted transcriptome analysis using RNA sequencing (RNA-Seq) in liver tissues obtained at autopsy from 30 criminal casework cadavers in Italy and the United States. Fifty nanograms of total RNA were converted to complementary DNA (cDNA) using reverse transcriptase and random primers. Targeted Oligonucleotide Pool (TOP) was hybridized to the cDNA on a streptavidin bead matrix. The resulting libraries were sequenced on the MiSeq<sup>®</sup> instrument. Percent contributions of liver biomarkers versus Postmortem Interval (PMI) for the overall set were determined and graphed. The results demonstrated that in each of the cadavers, the tissue was correctly identified as a liver sample. Furthermore, 98%–100% of the reads were attributable to liver biomarkers (e.g., AMBP, AHSG).

In conclusion, the results demonstrate as a proof of principle that a specific 8-biomarker liver assay is capable of identifying the original source of liver specimens from using cadaver tissue. The results also confirm that RNA molecules are stable in postmortem liver samples up to seven days, which makes RNA a sufficient molecule for gene expression studies. The design of this study validates a technique that will meet the demand for rapid and reproducible postmortem transcriptome methods to identify tissue fragments present in a variety of medicolegal investigations.

#### Criminal Cases, RNA Sequencing, Organ Tissue Identification



### **B113 An Analysis of Unusual Mutation Patterns in Father-Son Pairs Using a ForenSeq™ DNA Signature Prep Kit and a YFiler™ Plus Polymerase Chain Reaction (PCR) Amplification Kit**

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**Learning Overview:** The goal of this presentation is to present some unusual Y-chromosomal Short Tandem Repeat (Y-STR) mutations that led to discordance between a father and his sons.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by displaying examples of father-son discordance that, if not understood, could lead to wrongful convictions based on Y-chromosome analysis.

The application of Y-chromosome analysis is expanding in fields such as forensic science and genealogy. By researching the potential polymorphisms this chromosome can present, we can further our ability to assess DNA profiles for these disciplines to avoid erroneous exclusions of paternal linkage, wrongful convictions based on forensic evidence, and other misinformed genetic conclusions. The conservation of Y-haplotypes during transmission occurs due to lack of genetic recombination events in the inheritance of the Y-chromosome (with the exception of the pseudoautosomal region). However, mutation events can occur on the Y-chromosome, resulting in haplotype changes. These changes can include duplications and deletions that may occur at Short Tandem Repeat (STR) or Single Nucleotide Polymorphism (SNP) loci used in forensic DNA analysis. These mutation events can become important in cases of sexual assault where male-female mixture samples have low amounts of male DNA such that the male signal is not amplified in currently used STR multiplexes and analysis using Y-STR loci is used.

This study analyzed DNA from a father and his 11 sons using two different methods for forensic genetic analysis; next generation sequencing and capillary electrophoresis. The DNA from Council on Education for Public Health (CEPH) /Utah Pedigree 1413 was obtained from the Coriell Institute for Medical Research, Hamden, NJ, in the form of frozen DNA extracts isolated from a blood lymphocyte cultured cell line. Sample DNA was tested with the ForenSeq™ DNA Signature Prep Kit using primer set A and the YFiler™ Plus PCR Amplification Kit. Using these two platforms, three Y-STR loci were discordant between the father and each of his 11 sons. At all three loci, the father possessed the same allele as the sons, as well as one additional allele. At two of these loci (DYS449 and DYS635), the additional allele was one repeat (4 bp) longer than that of the shared allele. At the third locus (DYS458), the additional allele was three repeats (12bp) longer than that of the shared allele. Following read count and peak height analysis, it was concluded that these double allele loci did not result from the presence of a single true allele and a corresponding n-1 stutter allele. With the knowledge that the DNA was extracted from a blood lymphocyte cell line, it was postulated that a somatic mutation may be present in the cell line. However, this study was not able to determine whether the mutations exist in the blood of the father (therefore, true somatic mutations) or occurred as a result of the cell culture process.

Details concerning the position of these loci on the Y-chromosome, the repeat motifs of the alleles, and the potential for duplication versus stutter as the originating event will be discussed. Potential locus duplications were compared to those reported on the STRBase list of allele variations and to information found in literature. The observed DYS635 locus had an allele designation of 21, 22, which is reported on STRBase. The DYS449 and DYS458 loci showed potential allele-specific locus duplications that were not found on STRBase. The implications of non-inheritable allele patterns in the Y-chromosome, such as this, can be significant when considering comparisons between DNA obtained from germline cells (sperm) versus a known sample obtained from blood or saliva.

#### **Y-Chromosome, Paternal Linkage, Locus Duplication**



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### **B114 Mitochondrial Analysis of Challenging Samples Utilizing the ForenSeq™ mtDNA Control Region Solution on the MiSeq® FGx**

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**Learning Overview:** After attending this presentation, attendees will comprehend the application of massively parallel sequencing technologies to mitochondrial control region analysis. Additionally, attendees will be given an overview of the advantages and limitations of massively parallel sequencing as it applies to data analysis and sample throughput for mitochondrial DNA (mtDNA) processing using the ForenSeq™ mtDNA Control Region Solution on a MiSeq® FGx.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the advantages of applying massively parallel sequencing to mitochondrial control region analysis. With scalable sample preparation and low limits of detection, this technology could make mtDNA analysis easier to accomplish for current analysts and more accessible to forensic laboratories that wish to expand services.

While Short Tandem Repeat (STR) genotyping is the best practice for human identification, mtDNA sequencing is a supplementary analysis with a great impact on forensic genetics. In some cases, including highly degraded bones, mass disaster remains, or rootless hairs, STR analysis cannot be used to positively identify a sample. This leaves important forensic questions that can be answered with mitochondrial genotyping. While not as discriminatory as STR typing, mtDNA analysis remains a powerful tool for forensic analysts in source attribution and identification. Despite the utility of mtDNA testing, it has been limited to few laboratories because it is labor intensive with high error rates and requires substantial time on valuable capillary electrophoresis instrumentation. Many of the limitations of Sanger sequencing can be overcome through the use of massively parallel sequencing technologies. As this newer instrumentation is implemented in forensic laboratories, it will allow for the expansion of forensic services to include mtDNA analysis of highly challenging samples.

This study utilized the newly released ForenSeq™ mtDNA Control Region Kit for the Illumina® MiSeq® FGx to genotype the mitochondrial control region of typical mock forensic samples, including buccal swabs, challenging bone samples, and rootless hairs. This study also tested the limits of detection for the assay as well as both manufacturer-supported library normalization protocols. This data was analyzed using the integrated ForenSeq™ Universal Analysis Software. Variant calls generated with this assay and software package were confirmed using traditional Sanger sequencing.

This assay is designed around multiple overlapping small amplicons that cover the control region of the mitochondrial genome. This chemistry is scalable for variable sample multiplexing allowing for high or low throughput based on analyst needs. Discriminatory mitochondrial profiles can be generated using as little as 1pg of input DNA, and the system allows for potential advances in the reporting of heteroplasmy in samples. All results obtained with massively parallel sequencing were concordant with those found with Sanger sequencing.

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#### **Mitochondrial Control Region, Massively Parallel Sequencing, MiSeq® FGx**



## B115 Investigator-Mediated DNA Transfer

Michelle Le, BS\*, Surrey, BC V4N 4S3, CANADA; Jason Moore, MA\*, Burnaby, BC, CANADA; Steen Hartsen, BS, Burnaby, BC V5G 3H2, CANADA; Georgina Jayne Lush, Burnaby, BC, CANADA

**Learning Overview:** After attending this presentation, attendees will better understand the challenges in assessing alleged contact DNA transfer events and complex DNA mixtures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how the high sensitivity of current forensic DNA technology enables the detection of an individual's DNA on an object they never directly contacted, which has the potential to produce falsely incriminating evidence.

Ongoing improvements in forensic DNA analysis technology allow increasingly small amounts of DNA to be detected. This had led to increased success in obtaining results from low template samples; however, research to evaluate the risks of passive DNA transfer has not progressed with the advances in technology. Because bias creates a tendency to associate a crime scene profile with direct evidence of criminal activity, there are significant risks in the lack of understanding of DNA transfer.

The purpose of this study was to explore the possibility of investigator-mediated transfer of contact DNA, based on a homicide case in which the defense raised this theory to question the presence of the suspect's DNA on the murder weapon. The officers at the crime scene reported that the coroner had handled a Lululemon bag, possibly containing the suspect's DNA, followed by the handgun he discovered on top of the bag, without changing gloves. The experimental design of this study modeled this alleged tertiary DNA transfer chain, from the Lululemon bag, to nitrile gloves, to a gun, following the procedure used by Fonnelop, Egeland, and Gill.<sup>1</sup> This was done having zero, one, two, and three individuals handling the gun prior to the transfer procedure, in order to examine the effect of background DNA.

DNA was extracted by the organic extraction and microfiltration method. The samples were quantified using the Quantifiler® Trio kit before amplification using the GlobalFiler® kit, with capillary electrophoresis performed using a 3500 Genetic Analyzer. DNA profiles that indicated the presence of a mixture were analyzed in STRmix™.

A shedder test was first conducted as per Goray et al. in order to estimate the shedder abilities of the four participants in the study.<sup>2</sup> One participant was selected based on their good shedder classification to act as the primary donor, or "suspect." The other three participants were classified as poor shedders, who deposited partial DNA profiles. One appeared to be a very poor shedder, who deposited more of another individual's DNA than their own.

In the first DNA transfer experiment, a full primary donor DNA profile was found on the gun in two out of three replicates, despite the fact that the primary donor never held the gun. A full DNA profile was found on the bag and glove in all three replicates. A repeated measures Analysis of Variance (ANOVA) with sphericity assumed determined that the mean DNA concentration was significantly different between the three substrates ( $F[2, 4]=10.022, p=0.028$ ). Mauchly's test,  $\chi^2(2)=5.341, p=0.069$ , did not indicate any violation of sphericity.

When one individual had handled the gun prior to the transfer of the primary donor's DNA, near full primary donor DNA profiles were found on the gun. There was very strong support for the inclusion of the primary donor's DNA in the mixture. In one sample, the DNA profiling results are 41 trillion times more likely if they had originated from the primary donor and three unknown individuals than if they had originated from four unknown individuals. Where two and three individuals, respectively, handled the gun prior to transfer, the primary donor's DNA was not readily resolvable from the mixture. The primary donor was excluded as a contributor in most three-person mixtures, but likelihood ratios provided limited support for their inclusion in four-person mixtures. Notably, there was support for the exclusion of the very poor shedder from the mixture, despite them directly handling the gun.

These results suggest that there is currently a paradoxical relationship between the expanded forensic DNA technological capabilities and the level of confidence that can be placed on the nature or origin of contact DNA samples and the inclusion of minor contributors in a complex mixture. This study provides evidence that low template contact DNA evidence should be interpreted with caution.

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1. Fonnelop, Ane Elida, Thore Egeland, and Peter Gill. Secondary and Subsequent DNA Transfer during Criminal Investigation. *Forensic Science International: Genetics* 17, (2015): 155-162.
2. Goray, M., S. Fowler, B. Szkuta, and R.A. van Oorschot. Shedder status—An Analysis of Self and Non-Self DNA in Multiple Handprints Deposited by the Same Individuals Over Time. *Forensic Science International: Genetics* 23, (2016): 190-196.

### DNA Transfer, Contact DNA, Complex Mixtures



### B116 An Assessment of Cell Suitability of Touch DNA Samples for Forensic DNA Profiling

Jessica Surratt\*, University of New Haven, West Haven, CT 06516; Claire L. Glynn, PhD, University of New Haven, West Haven, CT 06516

**Learning Overview:** The goal of this presentation is to inform attendees of the potential for nucleic acid binding dyes to be used as a screening method for touch DNA samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into visualizing touch DNA using nucleic acid binding dyes as a means of screening samples to assess suitability for further processing (i.e., DNA profiling). This will ultimately allow investigators to sample more areas suspected to contain touch DNA, thereby increasing the recovery of probative information.

Touch DNA, also known as trace or transfer DNA, is typically deposited on surfaces by a person touching an item and leaving behind cellular material. The origin of touch DNA is suggested to come from several sources. It is hypothesized that the DNA comes from nucleated cells from the hands or other parts of the body that have been touched, cell-free DNA from residual body fluids, and cell-free DNA from cells that have undergone apoptosis.<sup>1</sup> In forensic investigations, touch DNA is commonly collected from surfaces using the double-swab method or by collection using adhesive tape (e.g., mini-taping). As these cells are microscopic, the investigator is blindly sampling areas where it is suspected to have been touched. These sampled areas may contain little to no DNA, which is not realized until DNA extraction and quantitation has been performed. Therefore, a screening method to visualize the cellular material present would serve to assess if the samples contain sufficient cellular material for further processing. Nucleic acid binding dyes have recently come to light as a novel tool for this purpose. Nucleic acid binding dyes will bind to double-stranded DNA and can be visualized using fluorescence microscopy. There are several commercially available dyes that either intercalate between the nucleotides or bind to a groove in the DNA structure. This research investigates five different nucleic acid binding dyes on touch DNA samples collected using both the double-swab and mini-taping methods.

Following ethical approval from the Institutional Review Board (IRB), with informed written consent, touch DNA samples were deposited on different surfaces. A range of surfaces that are commonly encountered in forensic casework were tested, including white cotton, black cotton, blue denim, polyester, glass, ceramic tile, and wood. The methods used to collect the samples from the surfaces were mini-taping and the double-swab method. Eight different types/brands of swabs were investigated including cotton, polyester, and foam swabs. The dyes used were GelGreen® Nucleic Acid Gel Stain, EvaGreen® dye, Diamond™ Nucleic Acid Dye, RedSafe™, and SYBR® Green Nucleic Acid Gel Stain. Following collection, the swabs/tapes were dyed using each dye, and visualized using the Dino-Lite® fluorescent microscope with an excitation wavelength of 480nm and an emission wavelength of 510nm. Images were captured using the DinoXcope software (version 1.24). Samples treated with each dye were Short Tandem Repeat (STR) profiled to assess interference with profile generation.

The results of this research showed that all touch DNA samples collected from each surface using both collection methods were visualized using each dye. The mini-taping collection method provided more clarity in the images collected compared to the swabbing method, thereby allowing more optimal assessment of the cellular material present. The foam-tipped swabs were the preferred material of all the swab types as they produced the least background fluorescence. The Diamond™ Nucleic Acid Dye was shown to provide the strongest fluorescence compared to the other dyes; it is a cost-effective method, easy to use, and is non-mutagenic. All samples STR profiled produced full profiles.

This research highlights the potential of this novel screening tool to assess the suitability of touch DNA samples collected from evidence items and crime scenes prior to submission to the DNA profiling laboratory. This screening method will allow investigators to sample more areas for screening, with the purpose of submitting only the samples known to contain sufficient cellular material, thereby recovering more probative information.

#### Reference(s):

1. Burrill, J., Daniel, B., Frascione, N. A review of “Touch DNA” deposits: Variability factors and an exploration of cellular composition. *Forensic Sci Int: Genet* 2019;39:8-18.

#### Touch DNA, Nucleic Acid Binding Dye, Screening Method



### B117 How Sharing ChapStick® Influences DNA Profiles Obtained From the Rim of Ceramic Mugs

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**Learning Overview:** The objective of this presentation is to demonstrate the potential for simultaneous direct and indirect transfer from the lips to the rim of ceramic drinking vessels after the application of shared ChapStick®.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding to a growing body of research that influences the understanding and interpretation of transfer DNA evidence in medicolegal investigations.

DNA introduced to a crime scene through indirect transfer has become an area of concern in the forensic science community as increasingly sensitive technology continues to improve the detection of DNA unrelated to the criminal event, thus resulting in mixed profiles. A wealth of research on transfer DNA over the past decade has sought to better understand how the DNA within a trace sample was deposited on the surface where it was collected. It is accepted by the scientific community that DNA can be deposited by direct and indirect means on surfaces. The complexity of these transfer events can result in simultaneous direct and indirect transfer. The person contacting the object may transfer their DNA through direct contact as well as act as a vector for the indirect transfer of DNA not their own.

The rims of beverage cans, bottles, and glasses are commonly swabbed for DNA profiles related to missing persons cases or criminal investigations. While it is discouraged to share cosmetics for sanitary and health-related purposes, it is still common practice for lip balms to be used by multiple individuals. This study investigates the DNA profiles obtained from ceramic coffee mugs when lips covered in shared ChapStick® contact the rim of the drinking vessel. During the study, one participant (called the primary user) opened a new tube of ChapStick® in the morning, used it three times throughout the day, then shared the tube with a second participant (called the secondary user). The primary user then reapplied the ChapStick®. Five minutes later, both participants “drank” five times from an empty ceramic coffee mug, the rim of which was subsequently swabbed for DNA profiling to test the following null hypotheses: (1) a mixed DNA profile will not be detected in samples obtained from the mug rim of the primary ChapStick® user, and (2) a mixed DNA profile will not be detected in samples obtained from the mug rim of the secondary ChapStick® user. The DNA was purified from the swabs using the QIAGEN® QIAamp® DNA Mini Kit, quantified using Life Technologies™ Quantifiler® Trio, and amplified using Life Technologies™ GlobalFiler® Amplification Kit.

DNA profiles were generated from all of the samples. One sample produced a partial DNA profile consistent with the drinker. For 18.75% of the samples, mixed profiles were detected that were consistent with both participants. Two of these samples originated from swabs of the secondary user’s mug and one from the primary user’s mug. Therefore, both null hypotheses can be rejected. In all cases, the major profile was consistent with the drinker. There were no patterns detected regarding length of use of the ChapStick® (primary user versus secondary user) and indirect DNA transfer.

However, the results of this scientific study do demonstrate that the simultaneous direct and indirect transfer of DNA is possible from the lips of an individual who is wearing shared lip balm. Given that lip balm is a personal item that may be shared between individuals, it is important to show that a mixed DNA profile could be obtained after shared use.

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#### DNA Transfer, DNA Evidence, DNA Mixtures



### **B118 The Evaluation of New Field and Laboratory Techniques for the Recovery of Touch DNA From Handled Improvised Explosive Devices (IEDs) Rendered Safe and Post-Blast**

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**Learning Overview:** After attending this presentation, attendees will better understand the effects of new field techniques for pipe bomb disposal on the ability to recover a Short Tandem Repeat (STR) profile from touch DNA as well as the effects of detonation of a pipe bomb on touch DNA recovery.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees about how updated and improved field and laboratory techniques can impact the ability to recover DNA to identify individuals who assembled and/or handled IEDs.

Bombing incidents, most utilizing IEDs, account for an appreciable percentage of terrorist events across the globe. In order to counteract and prevent explosions, various methods are utilized by law enforcement in order to properly diffuse or dispose of these IEDs. In the case of pipe bombs, the explosion is caused by a buildup of pressure in the sealed pipe caused by the interior components undergoing chemical reactions with one another. To prevent this, methods are used to release the pressure build-up, therefore, stopping the explosion from occurring. However, past studies have shown that the methods utilized to render an IED safe can be detrimental to the ability to develop a DNA profile. Ideally, samples collected from the rendered safe explosives would yield touch DNA profiles left on the IEDs during assembly. As a separate issue, heat generated during deflagration can degrade this DNA again, making it difficult to determine the identity of those involved in assembly.

A new field technique utilizing a Nitro Turkey® shotgun round has been adopted by bomb squad units for the disposal of devices. In the past, both water and clay shot were used in order to try and ventilate the device. However, these methods caused the DNA evidence on the pipe to be lost or washed away to varying degrees, depending on the shot used. This study evaluated the effects of the new rendering safe technique using a Nitro Turkey® shot on the ability to recover DNA.

Pipe bombs ( $n=30$ ) of various materials (PVC and steel) were rendered safe utilizing the Nitro® shots, while an additional 15 pipe bombs of each material were assembled but not diffused. A dual swab technique using two swabs with 2% Sodium Dodecyl Sulfate (SDS) as the moistening agent was performed. Three samples were collected from each device, including separate samples from each end cap as well as the shaft. DNA was then extracted using a PrepFiler® Express BTA™ Forensic DNA Extraction Kit using an AutoMate Express™ DNA Extraction System. The DNA was quantified using a Quantifiler® Trio DNA Quantification Kit on 7500 Real-Time Polymerase Chain Reaction (PCR) System, amplified using a Fusion 6C Amplification Kit on a GeneAmp® PCR System 9700, and then genotyped using an Applied Biosystems® 3500 Genetic Analyzer. The quantity and quality of the resulting profiles obtained from the touch DNA were compared between undiffused pipe bombs and ones diffused using Nitro Turkey® shots for both pipe materials. These results will be compared to results generated in a previous study using the old rendering-safe techniques and less sensitive laboratory procedures. In addition, several steel pipes will be detonated in order to determine whether sufficient touch DNA remained on the pipe to generate an informative genetic profile utilizing either a traditional Capillary Electrophoresis (CE) -based approach or next generation sequencing using a MiSeq® FGx™ Forensic Genomics System. Preliminary data suggests that the rendering safe technique using the Nitro Turkey® shot does not have a significant impact on the amount of DNA collected from either steel or PVC pipe. This study demonstrates that newer applied laboratory methodologies and field techniques used for rendering IEDs safe may improve an analyst's ability to generate informative DNA profiles from rendered safe and conflagrated IED samples and highlights the need to continually reevaluate protocols used for genetic analysis of challenging sample types.

#### **Forensic Science, Touch DNA, Pipe Bomb**





### **B119 Forensic Identification: An Investigation to Corroborate Volatile and Biological Profiles for Subject Identification**

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**Learning Overview:** After attending this presentation, attendees will understand that the unique microbial composition on an individual's hand determines the person's Volatile Organic Compound (VOC) profile.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the potential for an idiosyncratic profile that combines chemistry (VOCs) and biology (DNA) as a viable resource for forensic identification. This resource/tool increases the potential for trace samples to discriminate between individuals at a crime scene.

Hands are an integral part in transferring cellular material, both human and microbial, to and from our surroundings. As a result, hands are significant in provenance investigations as specific microbiota can be deposited on everyday objects through touch interactions. Skin microbiome, including bacteria, fungi, and viruses, are unique to each person, and this "uniqueness" can be exploited and applied to forensic identification. Skin microbiota and VOCs are intrinsically linked due to microbial metabolic transformation of non-volatile organic compounds to VOCs that are characteristically represented in human scent. The ribosomal operons within bacteria are ubiquitously present and easy to amplify through Polymerase Chain Reaction (PCR) because of its highly conserved primer sites and plethora of phylogenetic information. Traditionally, the small sub-unit of the prokaryotic ribosome encoding the 16S rDNA gene is used to identify bacterial species. However, analyses of microbiota from touch interactions have proven to be difficult due to the low quantity of genomic DNA (gDNA) that can be collected and extracted.

Headspace/Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (HS/SPME-GC/MS) is commonly used to conduct VOC analyses of air and water samples. Previous studies have utilized HS/SPME-GC/MS as a forensic approach to examine VOCs collected from various regions of the body as a form of identification. The objective of this research is to analyze samples collected from the subject's palms to determine the relationship between the individual's bacterial genetic profile and the VOC profile. Palmar sweat samples and epithelial swabs were simultaneously collected for VOC and DNA analysis, respectively. DNA was initially screened using Length Heterogeneity Polymerase Chain Reaction (LH-PCR) to obtain an initial assessment of the bacterial community's diversity. Next generation DNA sequencing of the V3-V4 region of the 16S rDNA gene was performed to identify each individual species present. Preliminary data have also demonstrated the ability to provide human Short Tandem Repeat (STR) markers from the same samples that will enhance the information collected from corroborated profiles.

Whereas previous standalone touch DNA analysis and standalone VOC analysis has proven to be challenging and limited in the information it provides, concatenation of these combined data will allow for the advancement of human scent studies for forensic identification.

#### **Volatile Organic Compounds (VOCs), Human Microbiome, SPME**



### B120 The Reproducibility of Individual DNA Deposits Detected With Diamond Dye

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**Learning Overview:** After attending this presentation, attendees will have learned how differences in DNA shedding propensity can be tested using a nucleic acid stain and how stable individual shedding propensity is over multiple depositions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding information on different approaches to test for shedding propensity. Data on individual variation of how much DNA is deposited via direct transfer informs expert opinions on transfer probabilities.

Contact traces are an important part of DNA casework in all crime laboratories and, with optimized recovery, detection, and interpretation methods, many samples now lead to positive associations to a person of interest. The probative value of these associations and the possibility of passive transfer need to be considered carefully. It has long been established that there is individual variation on how much DNA is left behind when touching an item and at least on scientific study on transfer via handshakes concluded that it was “the relative shedding ability” of the two volunteers that had the largest effect on whose DNA was detected.<sup>1</sup> In 2018, Kanokwongnuwut et al. published an elegant method for testing shedding variability.<sup>2</sup> This study aimed at reproducing their DNA staining/cell counting approach.

Volunteers were asked to deposit a left ring finger print on a clean glass slide, then wash their hands and, after waiting for 30 minutes while not touching anything, deposit a right finger print. Prints were stained with 20x Promega® Diamond™ Dye and read at the Fluorescein Isothiocyanate (FITC) filter setting (495nm excitation and 550nm emission) on a Nikon® Eclipse® E600 fluorescent microscope. Signal counts were scored at 100x magnification for three different 0.5 x 0.5mm squares. The method worked well and gave clear signals. Signal density for prints collected prior to handwashing (left ring finger) was often very high, which made counting difficult. This was not a problem for washed hands (right ring finger), where cell nuclei counts were much lower (average 24 +/- 14, n=10). Not unexpectedly, there was a lot of variation in cell counts between two independent collection events for the unwashed print; for ten volunteers, only four had counts within 20% of each other. Differences ranged from 8% to 81%. This was similar for washed finger prints, where only three data sets had a difference smaller than 20% and the range was 11%–105%. The latter was unexpected; after handwashing and not touching anything, cell counts should have been closer to each other. Ranking volunteers as heavy and low shedders produced similar results for both types of prints; the same three were the highest shedders and the same two the lowest, which means unwashed prints also represent shedder status. In conclusion: in our hands, the method was not as reproducible as described by Kanokwongnuwut et al., but showed a similar distribution of heavy, intermediate, and light shedders.

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#### DNA Shedding, Contact Traces, Cellular Material

### B121 The Detection of Cryptic Single Nucleotide Polymorphism (SNP) Variants for Enhancing Human Identification Capabilities

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**Learning Overview:** After attending this presentation, attendees will be able to appreciate the advantages of using Massively Parallel Sequencing (MPS) technology to improve human identification capabilities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the detection of additional rare SNP variants within a microhaplotype region can increase the power of discrimination of DNA evidence.

Microhaplotypes (microhaps) are loci with two or more SNPs within an expanse of 300 nucleotides associated in multiple allelic combinations.<sup>1</sup> These biomarkers have small amplicon size, ancestry informative alleles, no stutter, and lower mutation rates than Short Tandem Repeats (STRs), which make them useful for human identification, relationship testing, mixture deconvolution, and ancestry inference.<sup>2</sup> Sanger sequencing does not provide the *cis/trans* relationship between individual SNP alleles while MPS allows distinguishing the parental haplotypes by clonal sequencing of each individual DNA strand. The detection of rare (cryptic) SNP variants can potentially increase the power of discrimination of these markers and the ability to correctly infer the number of contributors to a mixture. This study explored the potential of a newly developed pipeline to identify rare SNP variants within microhaplotype alleles and their impact on human identification.

A MPS panel of 74 microhap loci was implemented and evaluated on the Thermo Fisher Scientific™ Ion Torrent™ S5™ system.<sup>3</sup> To expand the potential of microhaps to human identification, a custom bioinformatic pipeline was developed (Microhaplotyper\_CR\_v1.0), which uses an alignment-specific tool to enable the detection of the SNP defining the microhap loci while identifying additional SNP variants potentially present in between the SNPs that define the locus. These extra variants can be highly informative if detected as they are generally rare and consequentially increase the power of discrimination of the overall profile when present. To evaluate the performance of the pipeline, 18 single-source samples of African origin were selected and run on the Ion S5™ platform. All DNA samples were genotyped using the Microhaplotyper\_CR\_v1.0 pipeline, which was installed as a plug-in on the S5™ server. The output file identifies the alleles based on the SNPs that define the locus and also detects all other variants present within the amplicon. The frequency of the additional SNPs of interest was queried on the public database SNP (dbSNP), which includes allele frequencies of global populations from multiple data sets.

On average, this study identified eight additional SNP variants per each individual tested. Preliminary analysis showed that six rare SNP variants were observed only once within the sample set tested at a frequency of 0.1%–4.6% in African populations reported on dbSNPs. Eighteen different additional SNP variants were detected at high allele frequencies in African populations (10%–40%) and frequencies ranging from 1%–9% in other global populations, including Europeans, East and South Asians, and Americans. For instance, one of the identified SNP variants (rs79763993) was found at 19.4% in the African population, 0.01% in the European, 1% in the American, and undetected in East and South Asian populations. Comprehensive analyses on a set of more than 800 individuals from 14 global populations are ongoing to identify additional rare SNP variants.

Preliminary findings suggest that rare SNP variants are present within the targeted microhap regions and can be identified with the newly developed pipeline. Detecting these variants will increase the discrimination power of a profile and will increase the mixture deconvolution capabilities of this microhap assay.

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#### Microhaplotypes, Massively Parallel Sequencing, Rare SNPs

### B122 Lectin Blot-Based Profiling of Salivary Fluid Glycoproteins Distinguishes Different Patterns Among Individuals

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**Learning Overview:** The goal of this presentation is to show that salivary fluid glycoprotein patterns differ among individuals and how they can be detected by Sodium Dodecyl Sulfate (SDS) gel electrophoresis and Western blotting with lectin staining.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing new knowledge of the differences (polymorphisms) in salivary glycoproteins. This study may facilitate the identification of the origin of saliva samples.

It is hypothesized that individuals have unique profiles or patterns of carbohydrates on their salivary glycoproteins. It is further hypothesized that differences in salivary glycoprotein profiles may be used in forensics to identify the source of saliva. There are precedents in the available literature of inter-individual differences in protein carbohydrates. For example, ABO blood group antigens are carbohydrate structures present on salivary mucins. Furthermore, whether individuals can express ABO blood group structures in saliva is dependent on a second genetic system—the Secretor gene. It is proposed that there are other inter-individual differences in salivary protein carbohydrate structures to be discovered per previous work.<sup>1,2</sup>

The specific goal of this research was to define the patterns of carbohydrate structures on salivary glycoproteins from different individuals that were detected by selected lectins (carbohydrate binding proteins) and determine if individuals have distinct profiles that differ from others and that can serve as a means of identification.

In regard to methods, glycoproteins in salivary fluid samples were separated by Polyacrylamide Gel Electrophoresis in the presence of Sodium Dodecyl Sulfate (SDS PAGE) on the basis of size. Staining of the pattern of proteins after transfer to nitrocellulose with carbohydrate binding proteins (lectin blots) was used to characterize the pattern of the glycoproteins in the different proteins. This technique has been previously validated by researchers in other institutions and works well on salivary glycoproteins.<sup>1-5</sup>

Biotinylated lectins derived from plants were used on salivary fluid from eight individuals and were: UEA-I (*Ulex europaeus* I), PNA (peanut agglutinin, *Arachis hypogaea*), ECA (*Erythina cristagalli*), TL (tomato lectin, *Lycopersicon esculentum*), and STA (potato lectin, *Solanum tuberosum*).

The results obtained showed that generally each lectin reacted with a different pattern of protein bands so that the patterns were mostly distinct for each lectin for the different samples. When the staining patterns for the five lectins were compared side by side for all samples, the patterns were different. It was found specifically that UEA-I did not stain proteins in two samples, weakly stained one sample, and stained proteins in five samples. PNA stained one major band in four samples and multiple bands in four other samples. ECA only faintly stained six samples and reacted more strongly with six others. TL stained all samples, but one had a high molecular mass component absent from the others. STA stained high molecular mass bands of different size in three samples but not in three other samples.

The conclusion was that a panel of five lectins UEA-I, PNA, ECA, TL, and STA had different patterns of reactivity that distinguished saliva samples from different sources. This finding may be further investigated for forensic applications.

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#### Lectins, Saliva, Glycoproteins



### B123 Electrochemical Screening of Synthetic Cannabinoids

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**Learning Overview:** After attending this presentation, attendees will be able to understand the use of electrochemical techniques as screening tools in forensic science.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by advancing toward a micro electrochemical novel sensing method that will provide qualitative and quantitative information to enhance workflow in laboratories to speed up screening tests and analysis samples, reducing costs.

The proposed approach will allow on-site testing of synthetic cannabinoids supporting for non-invasive sample analysis with minimal volume at a low concentration as a rapid detection system. New screening tools in forensic science are of growing importance and will have implications in screening of new abuse drugs that pose a significant threat to the United States people and other countries. The collective term Synthetic Cannabinoids (SCs) addresses a drug group that doesn't have any resemblance to classical cannabinoids. The name has its origin in the effect that these substances exert on the central and peripheral nervous system. A large number of intoxications and some deaths have been linked with its use. Most new versions of SCs are chemically modified quickly and thus are not banned as illegal substances.

Newest generation SCs include N alkyl indole indazole derivatives. Usually, chemical analysis of cannabinoids can be accomplished by means of gas and liquid chromatography with mass detection. While these are definitive techniques, the high cost of chromatographic equipment should be considered by laboratory facilities. By contrast, electrochemical techniques could be used as preliminary screening tests because they use low cost equipment and miniaturized electrodes, require minimal sample amounts, and can be easily adapted to forensic science research. Moreover, the development of portable equipment is an attractive advantage. Although electrochemical techniques should be considered, there are some difficulties with electrochemical determination, such as high oxidation potentials. Another problem is contamination of electrodes with insoluble oxidation products. Both were overcome using Diamond Boro Doped (DBD) electrodes and optimizing an electrochemical procedure of cleansing. Thus, this electrochemically eco-friendly method was developed for the screening of synthetic cannabinoids. This was performed in a 100 $\mu$ L cell of home-built design. Analysis of cannabinoids was performed at concentrations between 0.1mg/L–1.0mg/L at pH 5.02 in an artificial saliva media by Pulse Differential Voltammetry (DPV) in cathodic and anodic mode. Peak potentials were determined for 11 synthetic cannabinoids. The same cell was used to obtain calibration curves in Acetonitrile/Tetraethyl Ammonium Perchlorate (CH<sub>3</sub>CN/TEAP 0.01 M) in low concentrations using DPV. Cyclic Voltammetry (CV) was also performed in CH<sub>3</sub>CN/TEAP 0.01M. At concentrations between 8–20mg/L of cannabinoids using Pipette Tip Solid-Phase Extraction (PTSPE) with another cell adapted for organic solvents, peak potentials were determined on drops of solution. Three micro methods were developed and applied to 25B-NBOMe.HCl, N benzyl piperazine; 1,3 chlorophenyl piperazine; NN dimethyl tryptamine, Cis 4,4 dimethyl aminorex, JW018, JW073; XLR 11, MAM220, and AB Pinaca and Fubinaca. All are known drugs of abuse in seized street samples. Absorption spectra were obtained for every compound tested. The use of these micro methods is possible as a presumptive screening method prior to identification.

#### Diamond Boro Doped, Microscreening, Synthetic Cannabinoids



### **B124 Optimal Extraction of Fentanyl Volatile Organic Compounds (VOCs) for the Development of Canine Training Aid Mimics**

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**Learning Overview:** The main objectives of this presentation are to teach attendees how VOCs are used for mimicking sent profiles as well as how proper canine training aid mimics are developed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees how training aids are used in police work to safely train canines on the scent profiles of illegal drugs. This training aid was developed to help train canines on the scent profile of fentanyl. Since fentanyl is such a potent and dangerous drug, many police agencies are fearful of training their canines due to the life-threatening risks. This training aid will not only take away those risks, but it will also safely prepare canines to alert to fentanyl while working. This will not only help limit the amount of fentanyl coming into our country or onto our streets, but it will also help to decrease the number of fentanyl-related overdoses.

Using canines as a way to detect controlled substances, such as illegal drugs, can be dangerous since trainers and handlers are working with a living biological detector. The danger mentioned can stem from the potency of the drug being detected as well as the health effects the drug can pose on the canine if the drug is inhaled. This led to the development of safe training aids that mimic the scent profile of illegal drugs. Training mimics have been developed for drugs such as cocaine, methamphetamine, and MDMA. This project investigates the development of a training aid mimic for fentanyl.

Prior studies have concluded that fentanyl is 100 times more potent than morphine. This potency can not only be dangerous for humans but for police canines as well. Since police canines work exclusively with their sense of smell, the inhalation of fentanyl can be fatal. It is for this reason that many police agencies choose not to train their canines to detect fentanyl. However, there are a few police agencies that are taking the risk to better prepare themselves against the fight with fentanyl. Current methods for training police canines to detect fentanyl pose a threat to the canine's lives by exposing them to pure fentanyl or assuming that the scent profile is similar enough to heroin and the canine will alert.

This study investigates the scent profile of fentanyl by using a Solid Phase Microextraction (SPME) Gas Chromatograph/Mass Spectrometry (GC/MS) method to detect the VOCs in the headspace of fentanyl. When using this method, specific VOCs are isolated and introduced to canines that have been trained on fentanyl. This was done with the expectation that canines will alert to the odor of these VOCs in the same way they would alert to fentanyl. Once the proper scent profile was detected, it was used to create a training mimic that can be used to safely train canines to detect fentanyl.

The goal of this study was to create a training aid mimic to safely train police canines to detect fentanyl. This study was completed to not only properly prepare police canines to detect fentanyl, but to then utilize those canines to decrease both the amount of fentanyl entering the country and the number of fentanyl-related overdoses.

#### **Fentanyl, Canine Training Aid Mimic, Volatile Organic Compounds (VOCs)**



### B125 The Forensic Analysis of Temporary Tattoos

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**Learning Overview:** After attending this presentation, attendees will better understand the physical and chemical characteristics of temporary tattoos, including transferable picture tattoos and freehand inks marketed toward both children and adults.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing examiners with information that will help them distinguish a temporary tattoo from a permanent tattoo, which may prove useful in investigations and identifications.

For thousands of years, cultures worldwide have used permanent tattoos for social identification and artistic expression. More recently, temporary tattoos have become more common and can potentially be used to identify individuals when biometric traits are unavailable. Transferrable picture tattoos are available for both children and adults, and inks to produce freehand, temporary tattoos are being marketed toward adults. The different types of temporary tattoos can be analyzed and distinguished from permanent tattoos and further distinguished from one another utilizing a variety of analytical methods.

Visual, microscopic, chemical, and spectroscopic methods were used to analyze the temporary tattoos. Visual examination consisted of evaluating the package ingredients listed and comparing them to alternate data sources made available by the manufacturer, such as MSDS, and observation of the tattoos/inks with an alternate light source at various wavelengths of radiation to assess fluorescence properties. Microscopic examination was conducted utilizing stereomicroscopy and brightfield microscopy in an effort to evaluate the dye distribution and printing patterns. Chemical extractions were performed in an attempt to isolate the dyes in the temporary tattoos and remove the colorless adhesive present. Spectroscopy, specifically Ultraviolet/Visible (UV/Vis) light spectroscopy and Fourier Transform Infrared (FTIR) spectroscopy, was employed in an attempt to identify the chemical composition of both the freehand inks and layers of the transferable picture tattoos. IR spectroscopy was used to analyze and compare different sections of temporary tattoo samples through the use of Attenuated Total Reflection (ATR) and Reflection-Absorption (RA) spectroscopy.

Some temporary tattoo companies do not openly disclose the ingredients present in their products, making it difficult to assess the composition of the temporary tattoos from packaging alone. Of the many temporary tattoo samples viewed with an alternate light source, only a handful of samples exhibited fluorescence, albeit weakly. The optimal conditions for fluorescence were found to be with a red filter and light with a wavelength of 555nm. Brightfield microscopy revealed that different printing patterns can be observed within one temporary tattoo sample and between samples from different brands. UV/Vis spectroscopy revealed that for the transferrable picture tattoos, the wavelengths of maximum absorption did mostly correspond with what would be expected for each color, with the exception of the secondary colors orange and purple. Seven out of ten henna samples shared an absorption band at 670nm, but there were otherwise few notable similarities in their spectra. ATR provided valuable information about the similarities and differences between the temporary tattoo samples. In general, temporary tattoos of the same type (such as henna or jagua) or same brand of transferrable picture tattoo had similar spectra, while transferrable picture tattoos from different brands had noticeably different spectra. IR spectral data was overwhelmingly characteristic of the adhesive layer, with individual dyes being undetected. RA results were consistent with the ATR data, with differences observed in spectral resolution.

The most effective ways to distinguish temporary tattoos from permanent tattoos and to differentiate between temporary tattoos are microscopy and IR spectroscopy. Specifically, the discriminating characteristics found in this study were the printing pattern of the transferable picture tattoos and the chemical composition of the adhesive layer of transferable picture tattoos. If a printing pattern is observed, the tattoo is likely temporary. IR spectroscopy can distinguish between henna or jagua, two common types of freehand temporary tattoos. IR spectroscopy can also yield results consistent with an adhesive, another indicator of a temporary tattoo. As a preliminary study, this research sets the stage for future studies in evaluating children's and adult temporary tattoos for forensic purposes.

#### Tattoo, Microscopy, Spectroscopy



### **B126 The Calculation of Calibrated Likelihood Ratios (LRs) for Glass Using a Multivariate Kernel Density Model: Introducing a User-Friendly Graphical User Interface (GUI)**

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**Learning Overview:** The goals of this presentation are to demonstrate the calculation of LRs for glass using a GUI and the interpretation of evidence using numerical values.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how this software will help forensic scientists calculate LRs easily for casework glass using this GUI.

Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS) for the quantitative elemental analysis of small fragments of glass evidence is considered the “gold standard” in the forensic analysis and comparison of glass.<sup>1</sup> The Organization of Scientific Area Committees (OSAC)-approved American Society for Testing and Materials (ASTM) E30 standard test method of analysis measures the concentrations of 17 elements and describes the comparison of glass samples collected from a victim or suspect to a known source. If the ASTM method results in no significant difference between the elemental concentrations of the recovered and the known source, a likelihood ratio can be calculated to better report the weight of the “match” between the glass samples. It has been previously reported that the use of a Multivariate Kernel (MVK) density 2-level model can be calibrated using Pool Adjacent Violators (PAV) that result in very low Rates Of Misleading Evidence (ROME), less than ~1% false exclusions and less than ~1% false inclusion rates.<sup>2,3</sup> Three glass databases were used to evaluate the performance of the LR; the first database includes 420 automotive windshield samples of known (authenticated) sources, the second database includes 385 glass samples from casework (the Federal Criminal Police Office of Germany [in German: Bundeskriminalamt, BKA]), and the third is a combination of the two. In addition to low ROME rates, the calibrated LRs limited the magnitude of the misleading evidence, providing only weak support for the incorrect hypothesis. Finally, most of the pairs found to be “falsely included” were explained by similarity of manufacturer of the glass source.

This presentation will focus on the simplification of the application of the previously developed R code for routine calculation of the LR in casework. One of the disadvantages in utilizing R programming is that it requires considerable knowledge in using the R software. The aim of the current effort is to develop a GUI to access the in-house written R code to calculate LRs to interpret the forensic glass evidence. The current R code has five main sections including: (1) installing and loading the required R-packages; (2) loading the background database (any glass database with the log<sub>10</sub> of elemental concentrations of glass); (3) calculation of LRs for the samples in the background database (same source and different source) using the combination MVK+PAV calibration model; (4) load the casework data to be considered; and (5) calculate and calibrate the LRs obtained for casework data. All of the above steps within the process will be presented as graphical widgets within the GUI interface. The calculated LRs will be displayed in the “output” interface and the data will be saved as a “.csv” file. This presentation will include instructions on how to access the original R code, the simplified GUI interface with detailed instructions, and also make the background databases available.

The goal of this effort is to reduce the computational barriers to calculate LRs using the previously published MVK+PAV calibration model so that forensic practitioners can routinely use a quantitative and objective method for reporting glass evidence comparisons using an LR and couple verbal statements with a numerical interpretation of the casework evidence.

#### **Reference(s):**

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#### **Glass, Likelihood Ratios, GUI**





### **B127 An Evaluation of Materials for Documenting Injuries From Blunt and Sharp Implements**

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**Learning Overview:** The goal of this presentation is to provide forensic science practitioners with a direct comparison of materials used in making impressions for documenting injuries from blunt force and sharp instruments. This presentation equips forensic scientists with cost-benefit insight that may help them in deciding which materials may be most suitable to use in different circumstances, given access and availability.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with insight into different materials that can serve investigations in areas where expensive materials may not be accessible. This presentation will enhance their ability to evaluate alternatives when silicon-based impression materials are not an option, unavailable, or do not fit the needs of the forensic scientist.

Collecting and preserving tool mark impression evidence is critical for forensic autopsy. This evidence is often used to identify weapons used in homicide as well as to demonstrate the cause of death in legal settings. Medical examiners must use impressions to determine the mechanism of an injury and to identify the weapons used to cause those injuries. While the collection and preservation of evidence is an important part of field forensics, there is tremendous variation in the cost and variability of materials to make these impressions.

This investigation provides forensic science practitioners with a direct comparison of materials used in making impressions for documenting blunt force and sharp instrument injuries. This study tested the hypothesis that there are less expensive and easier-to-obtain materials that are just as good as expensive materials in documenting pattern injuries from blunt and sharp implements. The methodology used four implements to test across three different materials. The three different materials were: AccuTrans™ Casting Silicone (\$135 for 10oz), Polyform Model Air by Polyform Products, Inc. (\$12.49 for 16oz), and Mold Putty by Alumilite Corporation (\$26 for 10.58oz). Each implement was tested in different dimensions and orientations. The substances were impacted and then photographed with measurements taken and changes documented over time. The differences in measurement and aesthetic appearance were calculated and compared via photography. Measurements were taken daily for the first week, then again on day 14 and day 21. The results showed that all tools and material compounds showed variability in the first seven days. The data revealed that stable measurements could be obtained for all materials after two weeks when little variability was seen in any of the materials.

Based upon the data, the AccuTrans™ Casting Silicone provided the most stable and consistent measurements with low variability for some blunt force and sharp instruments. Inspection of the molds also showed extra detail on the AccuTrans™ Casting Silicone and the AccuTrans™ Casting Silicone appeared to be the most consistently accurate impression material overall. Surprisingly, the much less expensive Polyform Model Air was almost as accurate and showed the same low variability after two weeks. The results indicate that the AccuTrans™ Silicone Casting is best suited for small surface areas as it can be difficult to use to record large or multiple injuries.

This study reveals that while expensive materials for making impressions documenting blunt force and sharp instrument provide the most stable and consistent measurements, much less expensive materials perform almost as well, depending on the injury impression required. The less expensive material could be particularly useful for blunt force or sharp instrument injuries covering a large area or where the impressions across a large area need to be made in proximity. Finally, this investigation provides attendees with insight into different materials that can serve investigations in areas where expensive materials may not be accessible. This study enhances the ability to evaluate alternatives when expensive impression materials are not an option, unavailable, or do not fit the requirements for measuring the injuries presented because of mitigating circumstances or cost concerns.

#### **Impression Materials, Blunt Instrument Injury, Forensic Molds**



### B128 An Analysis of Amino Acids in Latent Fingerprints Using Gas Chromatography/Mass Spectrometry (GC/MS)

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**Learning Overview:** The goal of this presentation is to inform attendees of the method developed for removing latent fingerprints from a glass surface and the subsequent derivatization and analysis of amino acid content of latent fingerprints using GC/MS. Attendees will learn how each step of the sample preparation and instrumental analysis is optimized and developed for a successful analysis. Attendees will be presented with the data obtained from the analysis of standard mixtures of amino acids as well as the data obtained from real latent fingerprint samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a method that can potentially be used at crime scenes for the analysis of amino acid content of latent fingerprints. The amino acid content has the potential to provide information about the suspects, such as gender, possible diseases, or even ethnicity, which can help narrow the list of suspects when a match is not found in the national database.

Latent fingerprints are formed by the deposition of perspiration and oils, contained in the ridges of the skin, on a surface. In the case of a crime, latent fingerprints located at a crime scene are developed, and the ridge patterns are compared to those of possible suspects or criminal databases for identification purposes. However, if no suspect exists or there is no database match, the developed fingerprint is merely saved within the database for future reference. Recently, scientists have demonstrated that fingerprints can provide more information about potential suspects than the physical pattern of their skin ridges. One study has demonstrated a qualitative relationship between the overall levels of amino acids and the sex of the individuals. Since amino acid levels in biological samples of individuals are influenced by many factors, such as ethnicity, sex, diseases, and dietary habits, accurate amino acid profiling could help to provide more physiological insight into the individual who has left the fingerprint behind at a crime scene. However, the procedures employed to date have not been successful in individual analysis of amino acids in fingerprints and are not readily applicable in everyday crime scene situations, and more practical procedures need to be developed and validated.

The purpose of this project was to develop a quantitative and feasible method for reliably removing and analyzing amino acids from latent fingerprints. In this study, the amounts of individual amino acids were measured in latent fingerprints after being deposited on glass slides. The deposited latent fingerprint was first swabbed from the surface using a Q-tip<sup>®</sup> soaked in a mixture of solvents (n-butanol:acetic acid:acetone:toluene:water), which was then transferred to a vial and washed two times with the same mixture. The wash mixture was then evaporated, and the proteins and peptides in the fingerprint were hydrolyzed with a 6 M HCl solution at 110°C overnight. The HCl solution was then evaporated, and the derivatizing reagent and solvent, N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and acetonitrile, were added respectively after purging the vial with nitrogen gas. The derivatization was performed at 60°C for four hours. The derivatized amino acids were then separated and identified using GC/MS. Identification was performed by comparing the mass spectra and retention times of the derivatized amino acids in the fingerprints to those of the derivatized standard amino acids. The developed method was tested for the analysis of amino acids in male and female latent fingerprints. To perform quantitative analysis, the same procedure was applied to the glass slides spiked with a mixture of standard amino acids at different concentrations. The hydrolysis step was omitted for the standard mixtures. The data obtained from the standard amino acids were used to plot calibration graphs, which were used to calculate the amount of identified amino acids in the fingerprint samples.

#### Latent Fingerprint, Amino Acids, Gas Chromatography



### B129 High-Throughput Screening of Drugs of Abuse Using a Robust Thermal Extraction Ionization Source (TEIS)

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**Learning Overview:** The objective of this study is to demonstrate the high level of sensitivity and robustness of this combined system for rapid and confident compound identification of drugs of abuse.

**Impact on the Forensic Science Community:** The abuse of opioids and other related drugs continue to pose serious public health and safety issues worldwide. As these substances continue to cause widespread intoxications resulting in fatalities worldwide, robust and comprehensive detection is critical to enable forensic laboratories to rapidly and accurately identify these substances. This presentation will impact the forensic science community by describing a method combining a TEIS coupled with a Mass Spectrometry (MS) system used for ultra-fast screening of drugs of abuse without the need for chromatography or extensive sample preparation. The combined system can quickly identify unknown drugs of abuse with a high level of confidence suitable for rapid forensic screening.

**Methods:** The TEIS was combined to an MS system for the identification of drugs of abuse. Upon integration of the source to the mass spectrometer, the source was heated to 285°C to volatilize the solvent injection, and a sample pump was used to draw the gaseous molecules toward the ionization region. Samples were injected via a microliter syringe into an injection port at the front of the top block for liquid standards or a slot between the two heated blocks for paper swabs. MS detection was performed in positive mode using a multiple reaction monitoring scheme to detect precursor and product ions of each of the targeted analytes.

**Results:** The use of the TEIS on a mass spectrometer enabled the rapid acquisition of MS data for high-throughput screening. First, drug-of-abuse standards were injected into the source and MS parameters were tuned. This process was used for each of the drug-of-abuse standards to determine the fragmentation pattern of each analyte and to appropriately select two product ions. Drug-of-abuse residues were tested by swiping a piece of paper on a dry surface onto which an unknown drug-of-abuse mixture was left to dry. The piece of paper was then inserted into two heated blocks of the TEIS and positive identification of the unknown drugs of abuse was accomplished by monitoring the thermal desorption profile in the form of an Extracted Ion Chromatogram (XIC) for two transitions monitored for each drug analyte. This process enabled the confident detection and quantitation of cocaine, amphetamine, and MDMA as the unknown compounds in the swab residue. Quantification of the drug residues present on a cell phone screen was also performed. The amount of the drug residues transferred to the paper swab was calculated using the area value for each of the XIC peaks resulting from the thermal desorption profile for each of the two transitions and solving for  $x$  using the linear regression equation. Using this method, the averaged values for cocaine, amphetamine, and MDMA were found to be 8.16 ng, 7.84 ng, and 5.53ng, respectively.

**Conclusion/Discussion:** Accurate identification and confident identification of low levels of drug residues is feasible using the extracted XICs based on the MRM transitions for the drug analytes. This demonstrates that the combination of Atmospheric Pressure Chemical Ionization (APCI) with thermal desorption is suitable as a fast sampling and screening method for trace analysis of drug residues. This approach shows potential application for trace analysis of drug residues from containers and parcels for high throughput security screening.

#### Drugs of Abuse, Mass Spectrometry, High-Throughput Detection



### **B130 Pepper Plants and Magic Mint—The Application of Ambient Mass Spectral Analysis for the Rapid Detection and Quantification of Psychoactive Compounds in the Complex Matrices of Plant-Based Legal-High Substances**

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**Learning Overview:** After attending this presentation, attendees will better understand how mass spectrometric techniques can be applied to the identification of plant-based legal-high substances and the quantification of the psychoactive compounds in complex plant matrices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a protocol that will enable law enforcement and forensic crime laboratories to rapidly detect and quantify plant-based legal-high substances that are otherwise indistinguishable from plants that are non-psychoactive.

*Piper methysticum* and *Salvia divinorum*, commonly known as kava and salvia, respectively, are psychoactive plants identified by the United Nations Office on Drugs and Crime (UNODC) as two of the “20 plants of concern” containing psychoactive compounds. The non-characteristic appearance of these plants of abuse and their derived products make it difficult for law enforcement and forensic crime laboratories to distinguish them from plants that are not psychoactive or mind-altering. Current methods of analysis often include time-consuming sample preparation, lengthy run-times, overlapping retention times, and are heavy on the use of solvents. Although neither of these plants are federally classified as controlled substances, in states where they are scheduled or restricted, cases are rarely prosecuted because of the difficulties and challenges associated with proving the plant material to be what it is. The investigation presented here focused on resolving these problems with a method that circumvents the challenges associated with other methods, rapidly screens for unique chemical biomarkers of these plants, and quantifies their psychoactive constituents. Because of its unique attributes and features, the use of Direct Analysis in Real-Time High Resolution Mass Spectrometry (DART®-HRMS) as the instrumental approach assists in the accomplishment these goals. DART®-HRMS is an ambient ionization technique that allows for the direct analysis of samples, rapid screening and identification of compounds, and semi-automated quantification with the use of a linear rail system to produce consistent and reproducible results.

Representative kava and salvia samples were used to determine if DART®-HRMS could rapidly detect the relevant analytes of interest in these plants. The six major kavalactones and three flavokavains were detected in the kava products, and the compound salvinin A was detected in the salvia products. DART®-HRMS was then used to confirm that all 28 of these products originated from either kava or salvia plant material. Because yangonin and salvinin A are the major psychoactive components of kava and salvia plants respectively, standard curves developed using calibrators and internal standards of either yangonin-*d*<sub>3</sub> or cholesterol were used to quantify their presence in the various products. The ratio between the protonated yangonin and yangonin-*d*<sub>3</sub> isotope peaks was preserved in all calibrators, as was the ratio between the salvinin A [M+H]<sup>+</sup> and cholesterol [M+H-H<sub>2</sub>O]<sup>+</sup> peaks, and could therefore be used to develop standard curves to confirm their concentration in quality control samples. With the success of three runs for each of the psychoactive chemical standards, all validated according to the Food and Drug Administration (FDA) guidelines, the protocols were considered acceptable for application to commercial products with unknown yangonin or salvinin A content.

Eighteen kava products were purchased from eight vendors and consisted of powders, tinctures, capsules, roots, and pastes. Although two samples fell just below the validated curve range, the remaining 16 kava products contained yangonin concentrations that fell within the linear range of the standard curve. Tincture and liquid samples had yangonin concentrations that were between 1.03 and 4.59mg/mL, while the solid samples exhibited a range from 2.71 to 8.99mg/g. Eight salvia products were purchased from two vendors and included raw salvia leaf and enhanced leaf extracts of varying potencies. The salvia leaves had an average salvinin A concentration of 1.54mg/g, while the enhanced leaf extracts had concentrations ranging from 13.54 to 53.24mg/g depending on the reported strength of the extract (ranging from 5x to 60x). In addition, the concentration of salvinin A in the leaves and extracts were relatively consistent with previously reported salvinin A concentrations in *S. divinorum* products.

These results demonstrate the development of a protocol that can rapidly detect plant-based legal-high substances in a variety of complex plant matrices, in addition to enabling the quantification of the psychoactive components in the various sample types, and it circumvents many of the problems encountered during analysis of these plants by conventional methods.

#### **Psychoactive Substances, Plant-Based Legal-High Substances, Mass Spectrometry**

### **B131 A Trace Elemental Analysis of Aluminum Flake Particles in Automotive Metallic Paints for Traffic Accident Investigation Using Synchrotron Radiation Micro X-Ray Fluorescence Spectrometry ( $\mu$ -SR-XRF)**

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**Learning Overview:** The goal of this presentation is to establish a new method for forensic identification of automotive metallic paint fragments based on trace elements in the aluminum flake particles by  $\mu$ -SR-XRF.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by revealing that the trace elements in aluminum flake particles obtained by  $\mu$ -SR-XRF at SPring-8 became new indicators for the discrimination of automotive metallic paint fragments.

Automotive paint fragments are very important samples for traffic accident investigations. In forensic science laboratories in Japan, automotive paint fragments have been analyzed by the following four techniques: optical microscopic observation, microspectrophotometry, Fourier Transform Infrared (FTIR) spectroscopy, and Scanning Electron Microscopy/Energy Dispersive X-ray Spectrometry (SEM/EDS). However, there are cases in which the paint samples cannot be distinguished enough by these analytical methods. On the other hand, metallic pigments are widely used to enhance the designability of cars. It is known that aluminum flakes for automotive metallic pigments have a purity of 99.0% or higher and contain a large variety of trace elements. In this point of view, after shape observation and major element analysis of the aluminum flake particles in automotive paint fragments with SEM/EDS, the trace elemental analysis by  $\mu$ -SR-XRF was carried out using SPring-8, the world's largest synchrotron radiation facility. This study attempts to discriminate automotive paint fragments by the trace element composition of aluminum flake particles. The goal of this presentation is to establish a new method for forensic identification of automotive metallic paint fragments based on trace elements in the aluminum flake particles by  $\mu$ -SR-XRF.

Twelve metallic paints obtained from accident vehicles were collected for this research. For measurements of the paint fragment samples, three 0.4mm long by 0.4mm wide fragments, including metallic pigment layers, were separated individually with a scalpel blade. After carbon vapor deposition onto the separated fragments was conducted using carbon coater, SEM/EDS analysis was performed. The  $\mu$ -SR-XRF analysis was carried out at BL05SS of SPring-8. Since trace elements in the particles could be effectively excited, an incident X-ray beam of 20keV with a size of  $2\mu\text{m} \times 2\mu\text{m}$  was prepared by means of the Kirkpatrick-Baez (K-B) mirror system.

Based on the results of SEM/EDS analysis, aluminum (Al), silicon (Si), sulphur (S), and barium (Ba) were detected and the 12 samples were classified into 2 groups: Al-Si type (6 samples) and Al-Si-S-Ba type (6 samples). S and Ba are derived from a pigment dispersant ( $\text{BaSO}_4$ ), which can easily and finely disperse aluminum flake particles in reigns of car paints. Many elements, such as Al, Si, S, chlorine (Cl), calcium (Ca), Ba, iron (Fe), copper (Cu), zinc (Zn), gallium (Ga), lead (Pb), strontium (Sr), zirconium (Zr), and niobium (Nb) were detected in the aluminum flake particles by  $\mu$ -SR-XRF. Ga is generally contained in aluminum alloys because it is the same family element of Al. For that reason, Ga was detected in all aluminum flake particle samples. The aluminum flake particles could be identified with high accuracy by comparing the trace elements. It was found that the trace elements became new indicators for forensic discrimination of automotive metallic paint fragments.

#### **Aluminum Flake Particles, Automotive Metallic Paints, Synchrotron Radiation X-Rays**



### **B132 Optimization of the Forensic Identification of Blood Using Surface-Enhanced Raman Spectroscopy (SERS)**

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**Learning Overview:** After attending this presentation, attendees will understand the principles of SERS, the characteristic spectroscopic features for blood identification, the wide variety of substrates on which SERS testing can be applied, and the ease of use and practical application for in-field sample analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a universal technique for the practical application of the identification of blood using SERS. It is recommended that crime scene investigators and detectives become familiar with this technique for the quick and easy use of on-scene sample analysis.

Blood is considered one of the most important forensic evidence found at a crime scene. The use of SERS provides a non-destructive and highly sensitive technique for the confirmation of blood for forensic relevance and can be applied on a portable Raman device for quick sample preparation and processing.

SERS is one of the few confirmatory techniques for blood and the only spectroscopic technique employed for the identification of blood at a crime scene or in the forensic laboratory. This method is able to distinguish between blood from other body fluids by collecting the SERS spectrum on a substrate surface that has been embedded with gold nanoparticles (AuNPs). The AuNPs create an electric field surface enhancement that produces an intense signal, leading to a SERS enhancement. The SERS enhancement allows for sensitive blood detection at dilutions down to 1:10,000, when dried bloodstains are not visible to the naked eye, mimicking a crime scene that has been previously cleaned. A stain transfer method to the SERS substrate was optimized by extracting dried bloodstains with water, saline, and 50% acetic acid solution. Acetic acid proved to be the most efficient in retaining the blood components and releasing the hemoglobin component of blood for detection.

The SERS spectrum of blood is a robust signature of hemoglobin that does not change over time. Characteristic peaks for the identification of blood are 754, 1513, and 1543  $\text{cm}^{-1}$ , attributed to a pyrrole ring breathing mode (Nu15) and two  $\text{C}_\beta\text{-C}_\beta$  stretches (Nu11, Nu38), respectively. These key SERS peaks, high sensitivity, and signal enhancement by a factor of ten are favorable when compared to normal Raman spectroscopy. A quick and easy procedure for in-field sample analysis for the detection of blood on different substrates has also been developed and applied on a portable Raman device. Various non-porous and porous substrates, including glass, ceramic tile, cotton, denim, fleece, nylon, acetate, wool, and polyester, have yielded strong results for the identification of bloodstains. In addition, testing different commercial and in-house SERS substrates has been effective in the identification of blood.

SERS identification of blood for forensic work is a non-destructive and portable tool that can be applied for quick and easy examination of evidence at a crime scene. The high sensitivity and selectivity of SERS provides a robust spectroscopic signature that aids in the confirmation of blood. It is a more favorable method when compared to alternative presumptive tests for blood and can be applied to stains on a variety of SERS substrates and sample surfaces for universal testing.

#### **SERS, Body Fluid Identification, Forensics**



### **B133 A Chemical Analysis of Gunshot Residues (GSRs) for Investigative Leads and Reconstruction of Firearm-Related Incidents**

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**Learning Overview:** After attending this presentation, attendees will have learned about the capabilities of Laser-Induced Breakdown Spectroscopy (LIBS) to offer rapid and reliable information in firearm-related investigations and to compliment other confirmatory chemical analyses (Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy [SEM/EDS] and Gas Chromatography/Mass Spectrometry [GC/MS]).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing a practical, objective, and reliable method to modernize the analysis and interpretation of Firearm Discharge Residues (FDRs) in clothing and non-movable objects.

When a shooting incident occurs, materials and compounds originating from the ammunition are deposited on surrounding surfaces in the form of FDRs. These residues, which include Organic and Inorganic GSRs (OGSRs and IGSRs), can become essential pieces of evidence. For instance, when the question of suicide or murder arises, estimating the barrel-to-victim distance may play a critical role in the outcome of a case. Also, clothing, wounds, and other target materials are often inspected to determine an entrance or exit bullet orifice. Conventional methods used in the course of these investigations include colorimetric assays that react with organic compounds and heavy metal components present in the propellant and primer. However, these assays have severe drawbacks regarding reproducibility and selectivity; they are challenging to perform in non-movable objects and are partially destructive of the evidence. Bloody and dark-colored items can significantly diminish the efficacy of these assays. Furthermore, modernized ammunitions (non-toxic) have eliminated the use of the heavy metal compounds needed for some color reactions.

As a result, this study aimed to develop a novel approach that uses LIBS chemical mapping for shooting distance determination on blood-stained clothing, identification of FDR on substrates of interest, and identification of IGSR of standard and non-toxic ammunition. The central hypothesis of this research is that LIBS will provide an enhanced analytical technique for the detection of standard and non-toxic ammunition on target materials because of its ability to simultaneously detect multiple elements in the ultraviolet and infrared regions, in just a few minutes.

The LIBS methods were developed and validated for the analysis of GSRs on substrates commonly found during firearm-related crimes. Residues were analyzed off 133 fabrics, glass, drywall, and wooden samples. For the determination of shooting distance, a calibration curve was created using 15 white clothing samples (100% cotton) covered in human blood, shot at known distances of contact, 6 inches, 12 inches, 24 inches, and 36 inches. An additional ten samples, five pristine white and five samples covered in blood, were shot at unknown distances. Once data was collected, integration of peaks was performed on elements of interest, including antimony (Sb) (259.8nm), lead (Pb) (405.8nm), and barium (Ba) (493.4nm). Principal Component Analysis (PCA) and leave-one-out cross-validated Regularized Discriminant Analysis (RDA) were then performed on the elemental intensities and chemical profiles obtained from these integrations at 20 different locations from the bullet hole. For comparison purposes, the clothing samples were then subjected to conventional colorimetric testing. Color tests resulted in a misclassification of three out of ten shooting distances (30%), while the LIBS method correctly classified the distance range of all unknown testing samples.

For the identification of suspected bullet holes, a total of 21 substrates of different materials (glass, wood, and drywall) were shot from a close distance to simulate the scene of firearm-related crime. The GSR surrounding the bullet hole was transferred to an adhesive sheet to eliminate the need to transfer the substrates back to the laboratory. The adhesive samples were then analyzed by LIBS using a rapid spectral mapping method. Spatial distributions of IGSRs, including Pb and Ba, were used to determine if a bullet created the hole in the substrate. Furthermore, four standard ammunitions (Sellier & Bellot®, Winchester®, Remington®, and TulAmmo®), and four non-toxic ammunitions (Fiocchi®, CCI®, Syntech®, and Inceptor®) were shot into textiles in multiple replicates, then analyzed by LIBS, SEM/EDS, and GC/MS for characterization of their chemical profiles.

Overall, LIBS allowed for rapid and accurate chemical mapping of GSR patterns on pieces of evidence typically found at a crime scene. Chemical imaging of Pb, Ba, and Sb provided more objective approaches to the estimation of shooting distance and bullet hole identification, compared to color tests. Moreover, LIBS, GC/MS, and SEM/EDS provided enhanced detection of standard ammunitions and lead-free ammunition.

#### **LIBS, Inorganic Gunshot Residues (GSR), Crime Scene Reconstruction**



### B134 Silicone-Based, Cost-Effective Alternatives to Traditional Casting Material for Large-Scale Impressions

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**Learning Overview:** The goal of this presentation is to present a method to create a new material for casting that will be a cost-effective, storable, and durable option for use in making large-scale impressions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that it is possible to create a cost-effective alternative to traditional casting materials for use on large-scale impressions, with considerations for in-field documentation and collection.

Traditional casting materials such as AccuTRANS™ and Microsil™ have been used successfully for many years to create casts of small impressions, such as those caused by tools. Larger impression evidence has been traditionally cast with products such as dental stone; however, its use is limited due to the weight and fragility of the resulting cast. In addition, storage and transport of a large cast can be problematic. While AccuTRANS™ and Microsil™ produce good-quality, fast-drying impressions that are lightweight, the cost of these materials may be prohibitively high if a large impression needs to be cast. Through the manipulation of different caulking compounds, in addition to various additives, it is possible to make a compound that is cost-effective, fast-drying, and retains a detailed cast without compromising the integrity of the impression substrate.

Three different types of caulking compounds were studied: 100% silicone-based compounds, acrylic latex-based compounds, and compounds comprised of a mixture of silicone and acrylic latex. Ten grams of each caulking compound were applied to a masonry brick with surface imperfections generated by a hammer and chisel. The caulking compounds were then allowed to dry completely and were then removed from the brick. Each caulking compound was evaluated on drying time, ease of removal, and impression quality.

After preliminary evaluations of the caulking compounds were completed, three classes of augmenting compounds were used in conjunction with each of the caulking compounds: spray-releasing agents, Monster Liquid Latex™, and Calcium Nitrate Tetrahydrate (CNT). The spray releasing agents were used to form a barrier between the casting compound being tested and the impression substrate in order to ease removal. It was determined that the use of spray-releasing agents greatly diminished the detail quality of the impression. The addition of Monster Liquid Latex™ at a ratio of 10:1 (caulk:liquid latex) increased the pliability of the casting compound; however, it also increased the drying time required. CNT was added to each of the caulk-liquid latex mixtures in an effort to reduce drying time while retaining the flexibility of the compound and quality of the cast.

To date, the best impressions have been obtained using a combination of 100% silicone caulk and liquid latex. It has been possible to obtain a detailed and reliable cast of the impression after a drying time of approximately three hours for the size of impressions used in the study. Research is ongoing to further reduce the drying time through the use of CNT while still producing a quality impression that retains integrity over time. Future research includes testing the optimized formula on a variety of substrates, temperature conditions, moisture conditions, drying time as a function of impression size, and assessing the scalability of the optimized compound.

#### Evidence Collection, Casting, Impression





### **B135 Integrating Undergraduate Mini-Research Project Exercises in Advanced Forensic Science Curriculum as a Course-Based Undergraduate Research Experience (CURE)**

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**Learning Overview:** The goal of this presentation is to discuss the utility of introducing students in forensic science programs to perform research, thereby enhancing their critical thinking skills and preparing them for a career in crime laboratories.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the community with future staff who have a better understanding of the fundamental knowledge of concepts involved in processing of evidence in crime laboratories.

One of the centerpieces of scientific education for undergraduate students with a science major is the incorporation of laboratory experiences.<sup>1</sup> The National Research Council (NRC) has emphasized the need for a revision in traditional laboratory courses to focus more on critical thinking skills and a deeper understanding and knowledge application.<sup>2</sup> More recently, original research and CUREs have gained a lot of attention as a high-impact strategy to improve learning outcomes resulting in improved student learning.<sup>3</sup> Additionally, several instructors have integrated research experiences into introductory science courses to improve undergraduate student interest and preparation for their science careers.<sup>4</sup> These research-based courses differ from expository (traditional) experiences since students are asked to develop procedures and outcomes for the experiments are not known. The benefits include learning important problem solving, critical thinking, communication skills, and gaining a deeper understanding of their field of study.

To improve student-learning outcomes in forensic science courses, this study has developed a research-based curriculum at the university. In the first seven weeks of the semester, students learn fundamental research techniques in a forensic biochemistry course that includes presumptive tests, DNA extraction, DNA quantitation, short tandem repeat-based polymerase chain reactions, and capillary electrophoresis. Using this fundamental knowledge, students develop a research problem/hypothesis, identify suitable protocols by a literature survey, plan and collect samples, determine variables, analyze data, and present their results as a formal laboratory report as well as an oral presentation. Students specifically design experiments dealing with changing variables (e.g., temperature, reaction conditions) in the collection, storage, and extraction of DNA for forensic DNA analysis. Data from Student Perception of Instruction (SPOI) and Student Assessment of Learning Gains (SALG) surveys administered at the end of the semester supported gains in student learning. Additionally, pre- versus post-survey data showed that students gained confidence in organizing and presenting their data, as well as a deeper understanding of the applications of biochemistry in forensic science. Incorporating research projects in other courses will help provide students with opportunities to be innovative and learn important critical thinking skills for their future careers.

#### **Reference(s):**

1. Elliott, M.J., Stewart, K.K., Lagowski, J.J. (2008) The role of the laboratory in chemistry instruction. *J.Chem Educ* 85(1) 145-149.
2. Fox, M.A. Hackerman, N. (2003) Eds. *Evaluating and improving undergraduate teaching in Science, Technology, Engineering and Mathematics*. National Research Council: Washington DC.
3. Murthy, P.P.N., Thompson, M., Hungwe, K. (2014) Development of a semester-long, inquiry-based laboratory course in upper-level biochemistry and molecular biology. *J.Chem Educ* chem 91, 1909-17.
4. Brownell, S.E., Kloser, M.J. (2015) Towards a conceptual framework for measuring the effectiveness of course-based undergraduate research experiences in undergraduate biology. *Studies in Higher Education*. 40,525-544.

#### **Forensic Education, Research, Mini Projects**



### **B136 Bode Armor™: A Developmental Validation of a Robust Preservative Solution**

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**Learning Overview:** After attending this presentation, attendees will gain an increased understanding of Bode Armor™, a preservative solution that can be applied to reference samples after collection to enhance sample stability.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing valuable data to laboratories that are looking to implement a solution to prevent degradation on their collected reference samples.

All 50 states in the United States collect DNA samples from convicted offenders and 31 states have legislation allowing for collection of DNA samples from qualifying arrestees.<sup>1</sup> The resulting DNA profiles may be entered into databases such as the Combined DNA Index System (CODIS). As of June 2019, the National DNA Index System (NDIS) contains more than 13.5 million offender profiles and more than 3.5 million arrestee profiles.<sup>2</sup>

When a hit or match is made in CODIS, the original reference sample taken from the individual is reanalyzed to confirm the genetic profile. This confirmation can occur soon after the sample is taken, or it can occur many years after the sample was originally collected and analyzed. As DNA is a biological material, cellular breakdown and degradation can occur over time if steps are not taken to preserve the sample. Degradation of the reference sample can cause interpretation issues if reprocessing needs to occur.

The adoption of any new method or technology requires careful consideration to ensure that it does not impact any downstream processing in the laboratory. This developmental validation evaluated Bode Armor™-treated reference samples and their ability to yield a complete DNA profile following both traditional processing (extraction, quantification, and amplification) and direct amplification procedures. This validation included the required studies for accuracy, artifacts, contamination, knowns, precision, repeatability, reproducibility, sensitivity, and stability. All experiments listed were performed using the THERMOFISHER™ GlobalFiler®/GlobalFiler™Express, Promega® PowerPlex® Fusion 6C, and QIAGEN® Investigator 24plex QS/24plex GO! amplification kits. Three amplification kits from three different manufacturers were chosen so that the resulting data would be valuable to most local, state, federal, or international agencies. All amplified samples were separated on an Applied Biosystems® 3500xL capillary electrophoresis instrument and analyzed utilizing appropriate analytical and stochastic thresholds for each amplification kit in GeneMapper® ID-X.

The resulting data showed that following manufacturer's recommendations, Bode Armor™ did not prevent the laboratory from obtaining accurate, reliable, and reproducible DNA profiling results. All DNA profile results obtained from Bode Armor™-treated samples were concordant with the previously obtained donor profiles.

The recommended application volume of Bode Armor™ is 100µl per reference sample. As expected, direct amplification procedures were impacted when the application volume was increased two- or three-fold. Traditional processing methods were not significantly impacted by the increased application volumes. Through this study, Bode Armor™ was shown to be a robust preservative solution that can enhance the stability of buccal reference samples while allowing for successful downstream analysis by DNA analysts.

#### **Reference(s):**

1. Hurst, Lisa. DNA Legislative Update. *18<sup>th</sup> Annual Forensic DNA Conference-Bode 2019*. Phoenix, AZ April 24, 2019.
2. <https://www.fbi.gov/services/laboratory/biometric-analysis/codis/ndis-statistics>.

#### **Bode Armor™, Databasing, Stability**



### **B137 Fiber Sampling Effects on the Detection of Fluorinated Coatings Studied by Pyrolysis-Gas Chromatography-Plasma-Assisted Reaction Chemical Ionization-Mass Spectrometry (Py-GC-PARCI-MS)**

*Michael J. Dolan, Jr., MS\*, Georgetown University, Washington, DC 20057; Wanqing Li, Georgetown University, Washington, DC 20057; Kaveh Jorabchi, PhD, Georgetown University, Washington, DC 20057*

**Learning Overview:** The goal of this presentation is to provide information on the suitability of fluorinated fiber coatings for fiber classification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees about the potential of fluorinated oil/water-repellant fiber coatings as differentiating characteristics for otherwise identical fibers.

**Introduction:** The value of fiber evidence is largely dependent upon commonality of fiber characteristics. Therefore, it is essential to utilize any differences possible, including fiber type, color, and chemical additives, to differentiate fibers. Fluorinated polymer coatings are a common chemical additive used to impart oil and water repellence for clothing. Previous work recently demonstrated that there are several classes of these chemicals that can be detected and distinguished using py-GC-PARCI-MS at a single fiber level.<sup>1</sup> In order to be useful in forensic analysis, it is necessary to also understand the variability and persistence of these coatings within sample collection. This work aims to address some of these forensically relevant questions.

**Methods:** Threads were pulled from commercial clothing items and single fibers were separated from the threads using tweezers. A single fiber was then transferred into a quartz tube for in-line pyrolysis. The pyrolysis products were separated by GC and fluorinated species were selectively converted to F<sup>-</sup> by the PARCI ion source for detection by a single quadrupole MS. Pyrograms were then compared pairwise by plotting the intensity of the peaks from one pyrogram vs. the intensity of the peaks from the other pyrogram. The correlation coefficients in the pairwise comparisons served as a similarity metric for the fibers.

**Results:** To examine location-dependent variability of coatings, a thread from five locations was sampled from two commercially coated clothing articles. The threads were analyzed in triplicate by testing three fibers from each thread. The fibers from various locations were examined for visual differences. Fibers analyzed from a multicolor shirt of uniform fiber type showed similar pyrograms, indicating that neither color nor sampling location had an impact on the detection of fiber coatings. Further, visually similar fibers from the second clothing item also supported the insensitivity of pyrograms to sampling location. However, for fibers from different locations that were also visually different, such as natural vs. synthetic fiber, differences in relative ratios of the pyrolysis products were observed. To further substantiate the utility of py-GC-PARCI-MS, this work will examine the persistence of fluorinated coatings in two experiments. First, determining whether contact between two clothing items can cause the coating to transfer from or to a fiber will be examined. Second, determining the effect of tape lifting, a common fiber collection technique, on the detection of fluorinated fiber coatings will be investigated.

**Conclusion:** This study has shown that the variability related to location in two different commercially coated clothing has minimal impact on fiber comparisons as long as only visually identical fibers are compared. Additional experiments are aimed at examining coating transfer between fibers and the potential impact of using tape lifts on fiber pyrograms. This information is critical in evaluating the potential forensic value of fiber coatings.

#### **Reference(s):**

- <sup>1</sup> Dolan, Michael J., Robert D. Blackledge, and Kaveh Jorabchi. 2019. Classifying Single Fibers Based on Fluorinated Surface Treatments. *Analytical and Bioanalytical Chemistry* 411, no. 19 (July 2019): 4775–84. <https://doi.org/10.1007/s00216-019-01596-6>.

#### **Fibers, Coatings, Sampling**



### **B138 Automated High-Throughput Potency Testing of Cannabis Samples Using Gas Chromatography/Mass Spectrometry (GC/MS) in a Forensic Lab**

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**Learning Overview:** After attending this presentation, attendees will be informed of a robust, automated approach to sample preparation for determination of total delta-9-Tetrahydrocannabinol (THC) in seized cannabis samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an avenue for increasing cannabis testing throughput to reduce case backlog and an approach to discriminate illegal marijuana from legal hemp.

In the United States, federal law and many state laws differentiate between marijuana and industrial hemp through delta-9 THC levels, whereby the latter is defined as less than or equal to 0.3 percent THC on a dry weight basis. Many traditional cannabis identification methods employed by crime laboratories cannot accurately discriminate between illegal marijuana and industrial hemp. Most current methods for determining total THC content in accordance with federal and state regulations do so with increased time, labor, and risks of instrument damage and compromised sample integrity. In the case of Richland County Sheriff's Department Drug Identification Lab, it was paramount to decrease preparation and analysis time for the fastest case turnaround.

GC/MS systems are often used by crime labs to identify other illicit substances, such as heroin. However, several challenges should be addressed when using GC/MS systems as a method to identify and quantify total available THC levels. GC/MS methods cannot detect the acidic Tetrahydrocannabinol (THCA-A) without decarboxylation or derivatization. In order to avoid the increased time and cost associated with derivatization, decarboxylation was evaluated *in situ* during GC/MS injection.

A novel, automated Dispersive Pipette Extraction (DPX) method uses tip-based technology and an automated liquid handler to enable fast, hands-free selective isolation of THC and its precursors for robust downstream GC/MS analysis. The method simultaneously processes up to 24 samples from test tube post-sonication through extraction and into GC vials in less than 11 minutes with no human involvement.

The clean extracts provided by the DPX method reduce the need for instrument maintenance normally resulting from matrix build up. The method provided an average of 86% recovery of THC. THCA-A to THC conversion in the GC/MS injection was evaluated to have a consistent average of 65%. The average precision values were below 3% Coefficient of Variation (CV) and the average accuracy values were above 98%. A blind study of non-probative case samples, acquired hemp samples, and previous proficiency tests was performed. Each sample was accurately classified as a result of this method. It has been validated and implemented by the Richland County Sheriff's Department Drug Identification Lab in Columbia, SC.

#### **Automation, Cannabis, THC Testing**



### B139 Complex Mixtures Made Suitable for Interpretation Using Probabilistic Genotyping

Christina Hayes Nash, MS\*, Bode Technology, Lorton, VA 22079

**Learning Overview:** This presentation will benefit attendees by providing real case examples in which probabilistic genotyping benefited the interpretation of data that would have been considered too complex to interpret using manual interpretation

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by assisting attendees in understanding the use of probabilistic genotyping in cases of complex DNA mixtures.

The increased use of megaplex Polymerase Chain Reaction (PCR) amplification kits has increased the occurrence of complex mixtures due to their increased sensitivity and the additional loci detected. Substantial major profiles are not always present for deduction, and manual methods for interpretation of mixture profiles containing more than two individuals often waste data that is otherwise robust (above stochastic thresholds). STRmix™, a probabilistic genotyping software that uses a fully continuous modeling approach for profile interpretation, allows for increased use of data.<sup>1</sup> It is based on a biological model, statistical theory, and computer algorithms. STRmix™ interprets DNA profiles from both single-source and mixed-source samples using a two-step approach; interpretation of DNA profiles, including deconvolution of mixtures if applicable, and then a likelihood ratio calculation to provide a weight to the possible match between the interpreted and reference profiles.

Forensic casework samples were processed in accordance with standard operating procedures at Bode Technology. The samples discussed will include clothing, firearm swabs, interior vehicle swabs, and sexual assault kit body swabs. Bode evaluated six cases to compare the suitability for interpretation with and without STRmix™. Approximately 70% of the profiles developed (8 of 11), were interpretable using STRmix™ but otherwise would have been unsuitable for comparisons and/or uninterpretable. The statistics obtained from these complex mixtures, which would have otherwise not been of any probative value due to manual interpretation confines, could be the critical factor in a case.<sup>2</sup> The forensic DNA community is moving toward increased use of probabilistic genotyping as a reliable mixture interpretation tool.

#### Reference(s):

1. Russel L, Cooper S, Wivell R, et al. A guide to results and diagnostics within a STRmix™ report. *WIREs Forensic Sci.* 2019;e1354. <https://doi.org/10.1002/wfs2.1354>.
2. Bille T., Weitz S., Buckleton J.S., and Bright J.A. Interpreting a major component from a mixed DNA profile with an unknown number of minor contributors. *Forensic Science International: Genetics* May 2019;40: 150-159.

STRmix™, DNA Mixtures, Statistics



### **B140 The Detection of Volatile Organic Compounds (VOCs) Released From Mass Storage Devices Utilizing Headspace/Solid-Phase Microextraction (HS/SPME) and Its Implications for Canine Training and Contraband Detection**

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**Learning Overview:** After attending this presentation, attendees will have more insight into the headspace analysis of various mass storage devices and the VOCs associated with them.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing recommendations for successfully training canines to detect mass storage devices.

Canines have been well known for their detection capabilities of illicit materials, such as explosives and narcotics. In recent years, canines have been trained to successfully recognize mass storage devices that may contain evidence of illegal activity. In cases of internet-based crimes, for example, evidence may be recovered as retrieved incriminating messages or files from the offender's mass storage device. These devices may be very small, as in the case of Secure Digital (SD) cards, and therefore very difficult to find as they may be hidden in areas not visible to the human eye. However, canines can be used to detect these clandestine devices by recognizing the odor (VOC) released by these devices. An example of this was observed in the home of former Subway® spokesperson, Jared Fogle, where a canine detected a hidden Universal Serial Bus (USB) drive containing incriminating pornographic material that was not previously found by investigators.

These canines have found regular usage within law enforcement correction facilities that employ them to find contraband devices being smuggled into the prisons. The method of training these canines for this purpose varies greatly, with limited scientific work done to validate the practice or determine the mechanism by which these canines detect these devices. As a result, theories as to the target material to be used for canine training are numerous, from training on entire devices for detection, such as a cellular phone, to training on only portions of the device, such as a lithium battery, creating even further variability and reliability in the detection capabilities of the canine.

The signature VOC and the specific odors that canines are alerting to in most of these devices remain to be scientifically determined. In addition, devices may produce different VOC profiles depending on the model, make, etc. This study analyzed a wide variety of cellular telephones, their components, such as batteries and Subscriber Identification Module (SIM) cards, as well as other commonly used mass storage devices. The results of this study will discuss the headspace VOC associated with these various devices and any common VOC that can potentially be used as a canine training aid to improve canine detection to a wider range of mass storage devices.

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#### **Canine Detection, Mass Storage Devices, Volatile Organic Compounds**



### B141 Indole and Related Non-Volatile Compound Release From Decomposing Mammalian Liver Homogenate

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the pattern of release of indole and some of the non-volatile compounds generated during decomposition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by clarifying the pattern of indole generation and release during the decomposition process. This knowledge could potentially be used in the determination of postmortem interval.

During the process of decomposition, biomolecules in the body are broken down via bacteria and other chemical reactions, most of which occur during the second and third stages, bloat and active decay, respectively. The transition period between these two stages is important because the release of the gaseous and liquid decomposition compounds from the body, or putrefaction, characterizes the end of the bloat stage and starts the active decay stage. The compounds produced throughout the process of decomposition include volatile, semi-volatile, and non-volatile species. Volatile compounds have been extensively studied and characterized using headspace Gas Chromatography/Mass Spectrometry (GC/MS), Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), and Time-Of-Flight (TOF) techniques.<sup>1</sup> The appearance of these compounds has been examined in relation to time, temperature, location, and other factors, with hundreds of unique volatile compounds having been identified over the course of numerous studies. However, an important issue regarding such volatile compounds is how long these compounds may remain or otherwise be detectable in the air surrounding the body, which is not only a question of time but also of weather conditions and other activity in the area. Relatively non-volatile or semi-volatile compounds, such as indole and similar species, in contrast, may be detectable near the body or gravesite longer, due to their lower volatility. Such compounds may therefore remain and be detectable to some extent, even after a body has fully decomposed.

Few studies have investigated the non-volatile compounds produced during decomposition. Prior studies on the non-volatile compounds of decomposition have noted the presence of fatty acids and cholesterol.<sup>2,3</sup> This study focuses on smaller, non-volatile compounds, specifically indole, that are produced by decomposition. Extracts of beef liver homogenate were utilized as the basis to analyze the non-volatile compounds obtained from these samples over a multi-day time course. GC/MS was used to analyze and qualify compounds extracted from the decomposing materials. Preliminary results confirm the presence of many fatty acids and cholesterol in the decomposed samples. However, also noted was the persistent appearance of indole in most of the decomposition samples, making it a prime candidate for future use as a marker compound for the decomposition process. Indole is an intermediate in the biosynthesis of tryptophan, as an intramolecular intermediate in the enzyme tryptophan synthase. Indole may be generated during the autolytic and putrefactive stages of decomposition by the action of bacterially generated tryptophanase. Indole, and related products from decomposition extracted from fluids, may provide information relating to the postmortem interval of decomposing bodies.

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1. Swann L.M., Forbes S.L., Lewis S.W. Analytical Separations of Mammalian Decomposition Products for Forensic Science: A Review. *Analytica Chimica Acta* 2010;682(1–2):9–22.
2. Swann, L.M. et al. Preliminary Studies into the Characterization of Chemical Markers of Decomposition for Geoforensics. *Journal of Forensic Sciences* 2010;55(2):308–14.
3. von der Lühse, B. et al. Investigation of Sterols as Potential Biomarkers for the Detection of Pig (*S. s. Domesticus*) Decomposition Fluid in Soils. *Forensic Science International* 2013;230(1–3):68–73.

#### Decomposition, GC/MS, Biochemistry



### **B142 A Simultaneous Chiral Analysis of Methamphetamine and Related Precursors and Screening of Methamphetamine-Related Organic Impurities in Seized Drugs by a Small Footprint Ultra High-Performance Liquid Chromatography-Photodiode Array/Mass Spectrometry (UHPLC-PDA/MS) System**

*Li Li, PhD\*, DEA, Dulles, VA 20166*

**Learning Overview:** The goal of this presentation is to present an efficient Ultra High-Performance Liquid Chromatography-Photodiode Array/Single Quadrupole Detection (UHPLC-PDA/SQD) method on methamphetamine profiling and chiral analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting an efficient UHPLC-PDA/SQD method on drug profiling and chiral analysis using a low-cost and small footprint system.

Methamphetamine (MA) is a widely abused stimulant of the central nervous system. MA can be synthesized by a number of routes starting from precursors of l-ephedrine/d-pseudoephedrine or 1-Phenyl-2-Propanone (P2P), which yield d-MA and racemic MA, respectively. Chemical profiling can be used to gather information about the manufacturing processes, emerging trends, cutting agents, and linkages between seizures. A number of analytical techniques are employed for the chemical profiling of MA samples. The detection of organic impurities and the determination of chirality for MA and its related precursors are essential to evaluate precursor chemicals and synthetic routes of clandestine MA production. Hence, dilute and shoot UHPLC-PDA/MS methods employing a small footprint and cost-efficient UHPLC-PDA/SQD systems are presented for the simultaneous chiral analysis of MA and related precursors (amphetamine, ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, methylephedrine, and methylpseudoephedrine), the targeted screening of MA manufacturing related organic impurities, and the untargeted screening of adulterants and emerging drugs.

Each pair of enantiomers was successfully separated in 9min by a LUX 3 $\mu$ m AMP chiral column with a mobile phase consisting of methanol and 0.2% cyclohexane. The Limit Of Detection (LOD) for MA by PDA, MS scan, and MS in Selected Ion Recording (SIR) mode, was 1 $\mu$ g/mL, 10 $\mu$ g/mL, and 10ng/mL, respectively. The chiral composition of MA was determined by using PDA data, even for samples with extremely skewed ratios of low enantiomer content. Route-specific compounds were separated in 8.5min by a BEH phenyl column with a gradient elution of acetonitrile and 0.1% formic acid. The SIR mode offered excellent selectivity and sensitivity (<25ng/mL) for the target impurities. Substances at a concentration of  $\geq$ 0.5 $\mu$ g/mL were detected by either PDA or MS scan. Using the developed methods, three sets of data (chirality, targeted, and non-targeted organic impurities) were generated from one sample set with two injections/separations of one sample preparation, which significantly improved the efficiency of MA chemical profiling. The developed method has been successfully applied to illicit MA samples, including tablets containing low doses of MA (<20%) and samples containing skewed ratios of d- and l-MA at trace levels.

#### **Methamphetamine Profiling, Chiral Analysis, UHPLC-PDA/MS**





## B143 The Differentiation Between Industrial Hemp and Marijuana Through Colorimetry, Spectroscopy, and Volatile Organic Compound (VOC) Profiles

Alexander Acosta\*, Florida International University, Miami, FL 33199; Jose R. Almirall, PhD, Florida International University, Miami, FL 33199

**Learning Overview:** After attending this presentation, attendees will better understand three different approaches to differentiating between industrial hemp product (<0.3% THC) and marijuana.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting a three-pronged approach to differentiate between industrial hemp and marijuana easily, rapidly, and accurately.

The 2018 Farm Bill permits the cultivation and legal trade of Industrial Hemp in the United States. Hemp is defined as *Cannabis sativa* and any part or derivative of the plant, including seeds, that has a Tetrahydrocannabinol (THC) concentration below 0.3% (w/w). There is now an urgent need to differentiate hemp from marijuana (THC content >0.3%) with a fast and easy-to-use analytical method. A three-pronged approach was investigated in order to find a suitable method(s) for differentiating between hemp (<0.3% THC content) and marijuana.

The first approach was the development of a semi-quantitative color test for the Fast Blue BB (FBBB) reagent and THC reaction producing an easily discernable red color above the 0.3% THC threshold. An extraction procedure was developed that allowed the plant material to be placed in an extraction tube without the need for weighing. In addition, the extraction solvent was able to be placed onto a Capillary Microextraction of Volatiles (CMV) device that was preloaded with FBBB reagent. A deep red color indicated a positive result and the CMV was then desorbed into a Gas Chromatography/Mass Spectrometry (GC/MS) for confirmation and quantitation of the chromophore formed between FBBB and THC.

Recent studies have shown the capability for near-infrared spectroscopy to detect and quantify cannabinoids from *Cannabis sativa* directly from the plant samples.<sup>1,2</sup> Hemp and marijuana were also analyzed using an Infrared (IR) -Tracer 100 Fourier Transform Infrared Spectrophotometer (FTIR) with a Near IR kit attachment (Shimadzu® Corporation) and with the SCIO®. Chemometrics techniques (Principle Component Analysis [PCA], Partial Least Squares Discriminant Analysis [PLSDA]) were used to extract the data from the Near IR spectra obtained and the data sets from the IR-Tracer 100 and the SCIO® were compared to each other. This comparison displayed the ability for hemp and marijuana to be differentiated using the SCIO®.

The third and final method involved differentiating hemp and marijuana using their VOC profile. A previous study in the Almirall lab reported variations in the VOC profiles of hemp products and marijuana.<sup>3</sup> For this study, industrial hemp was purchased from various vendors, and marijuana samples were obtained from local law enforcement. Dynamic headspace sampling was optimized and performed on hemp and marijuana samples to collect and preconcentrate the VOCs onto the CMV. Multivariate analysis was then used to observe if industrial hemp and marijuana could be distinguished using their VOC profile. The results show that marijuana and hemp can be distinguished in this manner.

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### Cannabis, Hemp, Near IR Spectroscopy



### **B144 An Analysis of Gaseous Mixture Adsorption by Nuclear Magnetic Resonance (NMR) Spectroscopy: Improving Our Understanding of Arson Debris Investigations**

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**Learning Overview:** After attending this presentation, attendees will learn how gas-phase NMR spectroscopy can provide valuable insight about the adsorbent properties of activated carbon by monitoring the real-time composition of gases in a sealed sample tube containing a carbon strip. Using this method, multiple compounds of interest can be detected and quantified simultaneously. In contrast to traditional approaches in which analytical techniques are used to determine what is desorbed after a solvent extraction of the carbon strip, this approach measures changes in the gas-phase composition to determine what has been adsorbed onto the carbon strip.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with exposure to a new method that allows for monitoring the rate of compound adsorption, the impact of water vapor on adsorption, and the role, if any, of competitive adsorption effects between gas-phase mixtures and carbon strips. These findings have the potential to improve our understanding of gas-adsorbent interactions.

NMR spectroscopy is most often associated with investigations of molecules in the liquid-phase. While not common, NMR is occasionally applied for the study of gases.<sup>1</sup> Recently, the National Institute of Standards and Technology (Boulder, CO) research group developed a method for the quantitative analysis of low-pressure gas-phase mixtures by <sup>1</sup>H NMR spectroscopy.<sup>2</sup> With this method, low uncertainty composition determinations (<1%) can be made and data collected in a matter of minutes. Interestingly, during these experiments, unexpected adsorption between gas-phase mixtures and a Teflon<sup>®</sup> piston located inside of the sampling apparatus were observed. While inconvenient at the time, these findings led the research group to explore the possibility of measuring mixture adsorption for other systems of interest by NMR. Here, the original approach to measure gas-phase compositions in the presence of a carbon strip is extended in this work. Data for individual compounds and simple binary mixtures are presented that demonstrate the features of this technique and its potential impacts on the field of arson debris analysis; other problems of potential interest to the forensic community are discussed.

To conclude, NMR spectroscopy allows for low uncertainty composition determinations of gas-phase mixtures. Using this technique, questions related to the kinetics and competitive effects during gas-phase adsorption can be explored. This approach could improve our understanding of the underlying science of arson debris analysis and other topics in forensic science.

#### **Reference(s):**

- <sup>1</sup> Jameson, C.J. Gas-Phase NMR Spectroscopy. *Chem. Rev.* 1991. 91: p. 1375-1395.
- <sup>2</sup> Suiter, C.L., McClinden, M.O., Bruno, T.J., Widegren, J.A. Composition Determination of Low-Pressure Gas-Phase Mixtures by <sup>1</sup>H NMR Spectroscopy. *Anal. Chem.* 2019. 3: p. 4429-4435.

#### **NMR Spectroscopy, Arson Debris, Quantitative Analysis**



### **B145 The Validation of Gas Chromatography With Flame Ionization Detection (GC/FID) and Development of a Method to Quantitate $\Delta^9$ -Tetrahydrocannabinol (THC)**

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**Learning Overview:** After attending this presentation, attendees will understand how to validate a GC/FID to detect and quantitate THC in marijuana samples. GC/FID is ideal for detecting THC due to its fast analysis and simple sample preparation. Currently, there is no method for quantitating THC at the District of Columbia Department of Forensic Sciences.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by furthering the methodology of quantifying THC using GC/FID.

Both hemp and marijuana originate from the plant, *Cannabis sativa*, differing only in the amount of THC contained within the plant.<sup>1</sup> Federally, marijuana is still a Schedule 1 controlled substance, whereas hemp has now been legalized.<sup>1</sup> Due to this distinction, quantifying the amount of THC in plant samples submitted to the laboratory is crucial.

Two GC/FIDs were validated following the procedures and validation process already in place at the District of Columbia Department of Forensic Sciences Forensic Chemistry Unit. Following instrument validation, liquid samples of various THC concentrations were analyzed using a quantitative method developed by the Forensic Chemistry Unit at the District of Columbia Department of Forensic Sciences. A calibration model was determined, then used to quantitate quality control samples prepared by fortifying oregano with known concentrations of THC. Extraction was performed by grinding the plant material with a mortar and pestle, using a sieve, then extracted with hexane. THC was successfully detected; however, the percent recovery was too low for quantification.

A marijuana sample was analyzed using the same method on the GC/FID using petroleum ether and hexane separately for the extraction. The GC/FID was able to detect THC without interference from other cannabinoids, but because the concentration of THC in the sample is unknown, the percent recovery could not be determined.

Future research will focus on obtaining quality control samples of marijuana with known amounts of  $\Delta^9$ -THC to quantify using the calibration model created in this study. Extraction methods should also be studied in order to increase percent recovery. Two different calibration curves should be developed so that one can be used for hemp samples and the other can be used for plant material with a higher concentration of THC.

#### **Reference(s):**

- <sup>1</sup>. Hewavitharana, A.K., G. Golding, G. Tempany, G. King, and N. Holling. Quantitative GC-MS Analysis Of  $\Delta^9$ -Tetrahydrocannabinol In Fiber Hemp Varieties. *Journal of Analytical Toxicology* 29, no. 4 (2005): 258-261. doi:10.1093/jat/29.4.258.

#### **$\Delta^9$ -Tetrahydrocannabinol, Quantify THC, Gas Chromatography**



### B146 The Easy Application of Chemometrics to Forensic Chemical Data: A Software Tool for Forensic Chemists

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**Learning Overview:** The goal of this presentation is to inform attendees about a free specialized software being developed in Europe that facilitates easier application of chemometric methods in forensic chemistry casework, such as classification or profiling of drugs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a new software tool that allows easy application of the chemometric methods most commonly found in literature to real casework involving chemical data.

In recent years, chemometric methods have been explored to help analyze and extract useful information from forensic chemical data. While these methods have seen some success, their utilization usually requires rather advanced statistical and data analysis skills, such as deep theoretical understanding of statistics and statistical software. For this reason, the application of these methods can be intimidating for many forensic chemists, despite the clear benefits they could provide. To help resolve this issue, a specialized and free software called ChemoRe is being developed in Europe.

The work on ChemoRe was started in 2018 as the European Network of Forensic Science Institutes (ENFSI) project titled “Steps Towards European Forensic Area (STEFAs),” funded by the European Union. The software tool, based on popular statistical programming language R, is intended to provide a simple but powerful graphical user interface for forensic chemists to allow them to design and apply in practice the most common chemometric methods.

To determine the applicable methods, a literature survey was conducted as part of the project.<sup>1</sup> While not intended to be a comprehensive review, this survey was used to determine the most common use cases of chemometric methods in forensic chemistry literature, with a focus on illicit drugs. Based on these results, ChemoRe has been designed to be as intuitive as possible to apply to the typical workflow of forensic chemistry casework. This sets it apart from other similar software that also implement the same methods that tend to be more open-ended and require more statistical and computational expertise from their users.

ChemoRe acts as a bridge for its users to the powerful features of the statistical R-language through an interface, implemented with the Shiny framework that allows the user to design chemometric pipelines by a few clicks of the mouse.<sup>2,3</sup> This pipeline can be saved and validated within the software and subsequently converted to a ready method that can be applied to new case data. Additionally, the results obtained can be appropriately visualized to support interpretation and reporting of the results. This should make it attractive to forensic chemists who seek to make use of chemometric methods.

In this presentation, the ChemoRe software is introduced by presenting the relevant background on its development, including the results of the literature survey and main technical features. Furthermore, realistic example applications are introduced that show how chemometric methods can be applied to casework using ChemoRe.

#### Reference(s):

1. M. Bovens, B. Ahrens, I. Alberink, A. Nordgaard, T. Salonen, S. Huhtala. Chemometrics in forensic chemistry—Part I: Implications to the forensic workflow. *Forensic Science International*, Volume 301 (2019): 82-90.
2. R Core Team (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
3. Winston Chang, Joe Cheng, J.J. Allaire, Yihui Xie, and Jonathan McPherson (2019). *shiny: Web Application Framework for R*. <https://CRAN.R-project.org/package=shiny>.

#### Chemometrics, Forensic Chemistry, Statistical Methods

## **B147 DNA Extraction and Profiling From Human Bone and Tooth Samples Exposed to High Temperatures: A Comparison of Current Techniques**

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**Learning Overview:** The goal of this presentation is to offer best-practice procedures for the isolation of DNA from burned human remains and to recover DNA useful for identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a predictive model to determine which incinerated human remains are likely to produce the best results for Short Tandem Repeat (STR) analysis.

In the identification of forensic cases, it is critical to obtain information on the victim's identity from burnt bone and tooth remains. When bone and teeth are burned, both their physical and chemical properties change significantly, which prevents anthropological evaluations and successful DNA profiling. Heat causes difficulties in the identification techniques in which bones and teeth are used, depending on the degree of temperature exposure.<sup>1</sup> Therefore, it is necessary to evaluate the results of observation and analysis, and the information obtained in a certain order, with the changes occurring in bone and teeth during the burning period. The identification of burnt or buried bones and teeth has always been a difficult process due to the diminished quality and quantity of DNA, and it is still a challenge.<sup>2</sup> Due to heterogeneity in DNA due to the heterogeneity within the bone, unfortunately, the only reliable method for obtaining DNA from highly degraded specimens, such as burnt bones and teeth, in the literature is still not available.

Because teeth and bones are the hardest tissues in the human body and known as the most abundant types of biological remains that are available in forensic cases, this study focused on the evaluation of the DNA extraction efficiency of burned bone and tooth samples and obtaining a DNA profile under different environmental conditions of exposure to temperature and time. In this study, 50 samples consisting of 25 bones and 25 teeth obtained from 22 females and 28 males aged between 18 and 71 years were used, and 50 samples of oral swabs belonging to the same persons were also used to compare current extraction methods, and the maximum temperatures at which DNA from bone and teeth can be obtained were determined. The collected tooth samples were healthy erupted third molars. All bone and tooth samples were divided into eight groups and exposed to certain temperatures, which were 100°C, 200°C, 300°C, 400°C, 500°C, 600°C, 700°C, and 800°C. Each group was treated at the determined temperature for 1, 5, 10, and 15 minutes. The control and burned samples were mechanically ground and subjected to DNA extraction and amplification. Based on the amplification data, it was not possible to obtain DNA from the teeth subjected to 400°C for 10 and 15 minutes, 500°C for 15 minutes, 600°C, 700°C, and 800°C for 5, 10, and 15 minutes. In the first temperatures and times, which were 100°C and 200°C, and one and five minutes, it was possible to obtain an amplification similar to that of the control samples; however, in most Short Tandem Repeats (STRs), the amplification was very low from 300°C for one or five minutes onward. These observations support earlier findings and indicate that even in teeth subjected to high temperatures and that were burned, it is possible to amplify the DNA; however, the data also show that it would be difficult to obtain an STR profile, which is probably due to the size of these regions that makes them more prone to degradation.<sup>3,4</sup> In this study, DNA extractions could be carried out in bone samples exposed to a maximum temperature of 190°C and tooth samples exposed to a maximum temperature of 400°C and a new modified extraction method was also proposed for increasing the effectiveness and efficiency in routine forensic genetic identification processes.

The results of this research provide a quantitative study in order to obtain a DNA profile from highly degraded bone and tooth samples and may increase the ability of forensic scientists to target the burned human bone and tooth remains to produce high yields of DNA, thereby increasing the probability of positive identification. Under the same or similar conditions, future research could extend these results by analyzing other potential regions of DNA for forensic identification.

### **Reference(s):**

1. Samsuwan, Jarunya et al. A method for extracting DNA from hard tissues for use in forensic identification. *Biomedical Reports* 9.5 (2018): 433-438.
2. Gamba, Cristina et al. Genome flux and stasis in a five millennium transect of European prehistory. *Nature Communications* 5 (2014): 5257.
3. Godinho, Ricardo Miguel, D. Gonçalves, and A.C. Valera. The pre-burning condition of Chalcolithic cremated human remains from the Perdigoões enclosures (Portugal). *International Journal of Osteoarchaeology* (2019).
4. Johnson, Rebecca N., Linzi Wilson-Wilde, and Adrian Linacre. Current and future directions of DNA in wildlife forensic science. *Forensic Science International: Genetics* 10 (2014): 1-11.

### **Burned Bone, Burned Teeth, Identification**

### **B148 DNA Analysis From Human Skeletal and Tooth Remains: A Comparison of the Recent Isolation Methods for Removing Polymerase Chain Reaction (PCR) Inhibitors**

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**Learning Overview:** The goal of this presentation is to offer best-practice procedures for the isolation of DNA and removal of PCR inhibitors from human burned remains and to recover DNA useful for identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a predictive model to determine which incinerated human remains are likely to produce the best results for the removal of PCR inhibitors during extraction procedures.

DNA analysis of human bone remains is a crucial process for identifying missing persons and individuals in cases of mass disasters and wars. In this study, the ability of microbial DNA isolation kits to recover amplifiable bone DNA and remove PCR inhibitors was compared to other common extraction methods. The generation of a DNA profile from bone samples is an important part of the identification process, both in cases of mass disasters and in cases of unidentified persons.<sup>1</sup> As bones and teeth are often the only biological evidence remaining after exposure to challenging environmental conditions, intense heat, certain traumatic events, and in cases in which a significant amount of time has passed since the death of the individual, the ability to purify reasonable amounts of DNA from such hard tissues is always beneficial.<sup>2,3</sup>

Because sampling procedures from hard tissues for genetic analysis is a destructive process, it is important to understand the environmental and intrinsic factors that will contribute to the preservation of DNA.<sup>4</sup> DNA extraction systems consisted of several methods and kits, including a standard organic extraction and a QIAamp® DNA research kit. A preliminary study was conducted to determine whether the reagents contained in the kits were contaminated with human DNA. The standard protocol did not result in consistently amplifiable DNA. The protocol was optimized by altering the digestion step. In addition to DNA recovery, each extraction method was tested to determine its ability to remove the calcium, collagen, and humic acid PCR inhibitors associated with buried and burned skeletal remains. Since lack of DNA amplification is a common challenge encountered with bone debris, successful amplification of nuclear DNA for each extraction method was compared to see which amplifiable DNA was most frequently recovered. The five extraction methods were tested on several remains of human skeletons, including bones and teeth, that had previously shown inhibition of PCR during DNA analysis. After comparing the human DNA isolation kits with organic extraction and a standard DNA extraction kit for inhibitor removal, the amount of bone DNA recovered and the success of nuclear DNA amplification, the effectiveness of the DNA extraction kits for use in bone remains was determined, as well as the most convenient extraction method. Improving the ability to interpret DNA results from challenging samples will be an important aspect of the future of the field. Finding ways to reduce the cost of genetic analyses will reduce delays and allow more samples to be processed. Finally, adequate training and funding must be provided to recognize that sound research based on the scientific method is the key to advancement in any field of forensic sciences.

#### **Reference(s):**

1. Samsuwan, Jarunya et al. A method for extracting DNA from hard tissues for use in forensic identification. *Biomedical Reports* 9.5 (2018): 433-438.
2. Godinho, Ricardo Miguel, D. Gonçalves, and A.C. Valera. The pre-burning condition of Chalcolithic cremated human remains from the Perdígões enclosures (Portugal). *International Journal of Osteoarchaeology* (2019).
3. Johnson, Rebecca N., Linzi Wilson-Wilde, and Adrian Linacre. Current and future directions of DNA in wildlife forensic science. *Forensic Science International: Genetics* 10 (2014): 1-11.
4. Gamba, Cristina et al. Genome flux and stasis in a five millennium transect of European prehistory. *Nature Communications* 5 (2014): 5257.

#### **Burned Bone, Burned Teeth, Forensic Identification**



### B149 DNA Profiling of Rootless Hair Shafts Utilizing Massively Parallel Sequencing and Bi-Allelic Assays

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**Learning Overview:** After attending this presentation, attendees will understand the potential for rootless hair shafts to be used as genetic evidence for forensic purposes. Additionally, the utility of assays that include loci amplicons smaller than those found in traditional Short Tandem Repeat (STR)-based amplification assays will be discussed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the potential use of rootless hair shafts as discriminatory genetic evidence. With additional samples identified as viable sources of probative information, additional samples can be successfully processed during routine laboratory operations.

Shed hairs are often underutilized as genetic evidence. These hairs are often passively lost and do not contain a hair root, where intact DNA is usually found. However, with advances in genotyping techniques and instrument technologies, the remaining hair shaft may be more viable as evidence than previously thought. Using naturally shed hair samples, STR analysis often fails to provide profiles. However, there remains the possibility of DNA that is both low template and highly degraded by the keratinization process of hair formation remaining in or on a hair shaft. This remaining DNA, though unusable for STR analysis, may still be impactful in the forensic setting. Utilizing alternative methods for processing these sample types may provide information that can still be highly discriminatory.

Currently, complementing unsuccessful samples with mitochondrial genome sequencing is the best method for processing challenging rootless hair samples. Mitochondrial DNA is inherited maternally, with the lack of recombination in mitochondrial inheritance results in low discriminatory power for resulting genetic information. This makes exclusion or inclusion of possible donors difficult in cases where potential sources are related or share mitochondrial haplotypes by chance. The current limitations using the mitochondrial genome suggest a need for additional sample processing strategies. With current advances in available amplification chemistries and instrumentation, additional processing options are readily available. These novel options can be used in tandem with already established methods to increase the discriminatory power of DNA extracts obtained from rootless hairs.

This presentation will cover a preliminary investigation into alternative methods identified as promising for the genetic analysis of rootless hair shafts. In this research, samples of hairs were collected with informed consent. The rooted portion of hair samples was then removed before extraction with the InnoGenomics® Hair Extraction Kit. Sample extracts were amplified using three methods. As a control, STR analysis was attempted with the GlobalFiler® PCR Amplification Kit. Alternative sample processing strategies include amplification with the retrotransposon insertion polymorphism marker-based InnoTyper™ 21 chemistry, which was then analyzed using capillary electrophoresis. An additional test method applied massively parallel sequencing using the MiSeq® FGx. Samples were processed using the ForenSeq™ Signature DNA Prep kit with primer set B, which includes a number of bi-allelic single nucleotide polymorphisms that can be used for identification, ancestry estimation, or phenotyping.

#### **Rootless Hair Shafts, Massively Parallel Sequencing, Bi-Allelic Markers**



### B150 Examining Various Methods to Extract and Amplify Degraded DNA

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of options available to improve profile results from forensic samples containing degraded DNA.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing results from an in-depth study of various DNA extraction methods and commercially available amplification chemistries for improved recovery and profile generation from degraded DNA.

In forensic casework, degraded DNA is encountered more frequently now that technologies have improved and allowed for increased detection of low-level DNA from decades-old cases. It can be difficult to generate full Short Tandem Repeat (STR) profiles from these cases due to DNA degradation. Traditionally, mitochondrial DNA (mtDNA) testing has been more common in cases of highly degraded DNA, especially when the evidence is a bone or rootless hair. However, mtDNA testing is a more lengthy and costly process and few labs have the capabilities to analyze mtDNA. For these reasons, it is important to examine whether nuclear DNA results can be improved by using these new methods.

In this study, hair (roots and shafts) were collected from multiple volunteer donors and extracted via four different methods: the QIAGEN® QIAamp® DNA Micro Kit, the InnoGenomics® Hair Extraction Kit, the MicroGEM forensicGEM™ Universal kit, and Protocol C from the Brandhagen et al. paper on nuclear DNA in hair shafts.<sup>1</sup> Samples were then quantified using Applied Biosystems® Quantifiler® Trio to determine quantity and level of degradation of DNA. Four different amplification kits were chosen to evaluate the DNA profiling method: Promega® PowerPlex® Fusion, Applied Biosystems® AmpFℓSTR® MiniFiler™, InnoGenomics® InnoTyper™ 21, and QIAGEN® Investigator® DIPplex. InnoTyper™ 21 and Investigator® DIPplex are not STR kits and were chosen due to their differences and possible improvements over the current STR systems. Both InnoTyper™ and DIPplex are biallelic and test for Retrotransposon Insertion Polymorphisms (RIPs) and Insertion/Deletion Polymorphisms (INDELs), respectively. The amplicon sizes in these two kits are smaller than the typical STR amplicons, increasing the chance that the primer target region will remain intact within degraded DNA and generate a profile where a traditional STR kit fails to do so. In addition to testing the kits' abilities to amplify the hair shafts and roots, other casework type samples were tested (bone, aged, inhibited, and degraded samples).

The sensitivity study in all four amplification kits resulted in optimal profiles with 0.25 to 0.5ng of DNA. In general, the hair roots had sufficient quantity and performed well in all amplification kits. While an improved quantity of DNA was obtained from hair shafts extracted with the Brandhagen et al. protocol over the other extraction methods, the amount of DNA was less than 5pg/μl and resulted in limited profile data with all amplification kits, including those with smaller amplicon size. However, the increased DNA yield will likely increase success rates from hair shafts for mtDNA testing. When sufficient DNA from degraded samples is available, the shorter amplicon kits have the potential to generate more complete profile results than STR kits, increasing the statistical power of degraded DNA evidence.

#### Reference(s):

1. Michael D. Brandhagen, Odile Loreille, and Jodi A. Irwin. Fragmented Nuclear DNA is the Predominant Genetic Material in Human Hair Shafts. *Genes* 9, (2018): 640, <https://doi.org/10.3390/genes9120640>.

#### Degraded DNA, Challenging Samples, Hair





### **B151 The Benefits and Burdens of Implementing Probabilistic Genotyping (PG) Software**

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**Learning Overview:** The goal of this presentation is to relate challenges and rewards encountered when implementing and using PG software in DNA casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information to assist laboratories planning to implement PG software as a tool for DNA statistical calculations. Laboratories may encounter an unexpected negative impact on productivity after implementation.

PG methods are becoming widely used for DNA statistical analysis and this will continue as these statistical software tools provide more information in a more consistent and unbiased manner than previous methods. The Harris County Institute of Forensic Sciences implemented PG statistical software in February 2019 following validation and training DNA analysts over approximately 12 months. Since then, an increase in the number of informative results obtained per sample tested has been seen. Although the initial dip in productivity was attributed to analyst learning curve, it also became clear that as the sample success rate has increased, a commensurate increase in turnaround times was observed.

Low-level mixtures and mixtures exhibiting degradation become increasingly difficult to interpret manually as the number of contributors increases. This complexity is due to both potential missing information and an exponential increase in possible genotypes with each additional contributor. PG software allows an analyst to provide statistical calculations to samples that previously could not be evaluated through a binary method and were reported as inconclusive, uninterpretable, or having the potential for allele drop-out. While PG allows the laboratory to provide more information and do so more objectively, it does require more time to analyze samples. The additional information increases the detail in the report and the length and complexity of technical review.

To quantitate the increase in informative results, this study reviewed the percentage of samples that could be compared to a reference sample before and after implementing PG. Informative results increased from 18.8% to 34.2% with PG, almost doubling the reportable comparisons ( $n=1,451$  and 1,183 samples, respectively). Prior to PG, the average turnaround time of a sexual assault case (from submission to release of the report) was approximately 77 days and after implementation, it increased to approximately 98 days. This is approximately a 27.3% increase in time to complete analysis. To combat the increase in turnaround time, measures to limit the number of samples reported may be incorporated, such as a Y-screen for sexual assault samples or a stop at quantification procedure.

Using a PG statistical software can increase the number of informative results that a laboratory can report. Without additional changes to speed testing and reporting, the additional comparisons will increase the amount of time required to complete a case.

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### **DNA, Probabilistic Genotyping, Productivity**



### **B152 The Use of Relationship Likelihood Ratios (LRs) as a Diagnostic for Probabilistic Genotyping: Validation and Casework Experience Through the Years**

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**Learning Overview:** The goal of this presentation is to illustrate how familial relationship LRs can be used as an additional diagnostic for probabilistic genotyping through validation and to highlight casework examples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping to implement familial relationship diagnostics in individual labs.

Since 2016, DNA Labs International (DLI) has been online with STRmix™ expert forensic software. Validated for use with the Applied Biosystems® AmpFℓSTR® Identifiler® Plus on the Applied Biosystems® 3130xl Genetic Analyzer, and now with the Applied Biosystems® GlobalFiler® and Promega® PowerPlex® Fusion 6C on the Applied Biosystems® 3130xl and 3500xL Genetic Analyzers, thousands of casework samples have been evaluated.

At DLI, STRmix™ is used to aid the analyst in deconvoluting a DNA evidence sample and develop an LR. An LR is a ratio of two probabilities giving a numerical value that shows strength of support for one scenario over another. In the case of DNA, in the simplest of terms, it is saying whether it is more likely to observe the DNA profile if it originated from the individual of interest than from an unknown individual. The software also develops several types of relationship LRs, including sibling, parent/child, half siblings, uncle/aunt/niece/nephew, and first cousins where it addresses if it is more likely to observe the DNA profile obtained if a person of interest is part of an evidence sample versus a relative of the person of interest. This is of interest to both the prosecution and defense as members of a family share more alleles in common than unrelated individuals and can, therefore, lead to potential false inclusions or exclusions of individuals. It became clear from a few specific casework samples that the relative LR table provided in the STRmix™ report can be indicative of the inclusion of relatives into the mixture in question and can falsely inflate the LR for a person of interest.

As a result, additional validation studies were performed involving mixtures made from multiple family trees using different amplification kits and instruments. Diagnostic ranges were developed for comparison of the unified LR to the sibling LR as well as the unified LR to the parent/child LR, as these were shown to be the most affected. This diagnostic range, in conjunction with other diagnostics and the LR itself, may indicate when a relative LR should be of noted concern and would necessitate requesting standards of primary relatives of the person of interest for comparison. However, the LR value can be dependent on the quality of the profile and the overall presence of the applicable contributor in that profile as well.

Casework examples highlighting possible familial relationships and what was done to address them will be presented. Additionally, the diagnostic ranges that DNA Labs International employs for relative LRs that were developed during validation and how they were determined will be examined. This will benefit attendees by providing information on ways the familial LRs developed by STRmix™ can be explored during validation and how it relates to casework.

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#### **Probabilistic Genotyping, Familial Relationships, Validation**



### **B153 Six Months to 30 Days in 24 Hours: A Laboratory's Journey to Meet Statutory Requirements Relative to Sexual Assault Evidence Collection Kit (SAECK) Processing**

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**Learning Overview:** After attending this presentation, attendees will better understand how legislative mandates relative to the forensic analysis of SAECKs may impact a laboratory's operations. Attendees will gain knowledge of the practical, operational, and financial considerations for implementing such mandates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight that may assist laboratories with meeting statutory requirements relative to turnaround time on the analysis of SAECKs.

A national dialogue emerged several years ago as a result of the discovery of thousands of SAECKs sitting untested in law enforcement agencies' evidence storage facilities. This prompted demand for legislative reform by advocacy groups, scientific agencies, and law enforcement nationwide. The subsequent reaction of many jurisdictions has been one of action; identify the scope of the problem in their respective jurisdictions and implement measures to reform current practices with the goal of ensuring such a situation never repeats itself.

In April 2018, the Commonwealth of Massachusetts passed an Act Relative to Criminal Justice Reform (CJR) containing many initiatives designed to overhaul the Commonwealth's Criminal Justice System. Included in the reforms were sections that addressed topics directly applicable to forensic analysis providers within the Commonwealth. Specifically, one section directed the Commonwealth to identify and test previously unsubmitted SAECKs while another outlined requirements for testing current and future kits.

An Act Relative to Criminal Justice Reform, Chapter 69, Section 214, mandated law enforcement agencies to submit to the laboratory all previously unsubmitted investigatory kits to the crime laboratory. To accomplish this, each agency had to complete an inventory of SAECKs within their custody. Additionally, the section further prescribed that all identified previously unsubmitted investigatory SAECK must be tested within 180 days of submission.

Chapter 41 section 97B ½ addressed the processing of current and future kits by virtue of the following mandates: all investigatory kits would be submitted to the crime laboratory and tested within 30 days. Additionally, a SAECK tracking system would be implemented statewide between June 30, 2019, and December 1, 2019.

While well-intentioned, the CJR Act posed significant challenges to address before the mandates could be effectively met. Massachusetts has several hundred law enforcement agencies, including local, county, state, and academic institutions. By statute, all of these agencies were now required to complete the inventory, report on any SAECKs within their custody that fit the criteria for testing, and submit all identified kits to the laboratory. However, the statute did not specify the logistics or ultimate responsible party for ensuring this task was completed. Additionally, given that the number of potential kits subject to the new legislation was widely unknown, determining an appropriate facility to store what could be a significant number of kits posed another potential obstacle.

The 30-day turnaround time mandate for testing was progressive and likely intended to provide both expeditious justice for potential defendants as well as forensic answers for survivors. However, at the enactment of this bill, the Massachusetts State Police Crime Laboratory (MSPCL) lacked the adequate personnel, space, and equipment necessary to meet this mandate. Further compounding this, the statute was effective immediately, meaning no transition period was contemplated to achieve compliance.

Criminal justice reform is not unique to Massachusetts; many other jurisdictions have or may soon have mandates related to SAECK processing. This presentation will discuss the challenges associated with the reforms enacted within Massachusetts and the steps MSPCL has taken toward meeting these mandates. Specifically, the initial process for determining what kits fit the criteria of "previously unsubmitted," as well as the coordination of the inventory, collection, and testing of said kits, will be detailed. Additionally, the challenge to meet a 30-day turnaround time, including identification of resources needed and the data to support requests for these resources as well as optimization of current workflow, will be discussed. Lessons learned from the perspective of MSPCL will be shared, as well as suggestions for other agencies facing similar scenarios will be presented.

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#### **Sexual Assault, Legislation, Testing**

## B154 An Exploration of EDTA Detection Within Forensically Relevant Blood Samples

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**Learning Overview:** After attending this presentation, attendees will be familiar with new presumptive and confirmatory techniques for the detection of disodium Ethylenediaminetetraacetic Acid (EDTA) within blood samples. Challenges encountered during the development of these methods will be discussed, as well as their limitations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing potential new methods for the presumptive and confirmatory detection of EDTA within blood samples, which can assist in the identification of “planted” blood evidence. Additional insight into possible techniques for the removal of hemoglobin from blood samples will be discussed as a side benefit to forensic biologists.

Detection of EDTA within blood samples has recently resurfaced in the forensic science community, especially as it pertains to “planted” evidence. Any method intended to distinguish authentic blood evidence from planted evidence requires the detection of exogenous component(s), such as EDTA, that would normally be absent in human blood. The ability of EDTA to form a stable complex with calcium, magnesium, and other metal ions enables its use during complexometric titrations to detect and quantify metal ions within a solution.<sup>1</sup> A colorimetric indicator, Eriochrome<sup>®</sup> Black T, was identified as a possible candidate for use in the detection of EDTA. This indicator changes color when free calcium and magnesium ions are present. If EDTA is present, it will preferentially bind these ions, preventing their interaction with the indicator. As visualization of a color change is inherently subjective and can preclude color-blind individuals from performing this protocol, use of UV/Visible (UV/Vis) spectroscopy could remove such subjectivity. It was hypothesized that the Eriochrome<sup>®</sup> Black T indicator, in conjunction with UV/Vis spectroscopy, could be used to presumptively detect the presence of EDTA. Furthermore, Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) and Raman spectroscopy were also explored as possible confirmatory methods for the identification of EDTA. These rotational-vibrational techniques were selected given their high potential for compatibility with EDTA and to supplement previous reports using other instrumentation, primarily chromatographic, for EDTA detection.<sup>2-6</sup>

To establish proof of concept, sensitivity studies were performed based on expected levels of EDTA added to human blood, as well as expected levels of calcium and magnesium normally occurring in human blood, while also factoring in forensically relevant blood sample sizes. EDTA standards (0.003–3mg) were utilized for all four methods, whereas calcium and magnesium standards (1–30 $\mu$ g) were also necessary for the colorimetric indicator test and subsequent UV/Vis. For all EDTA amounts tested, Eriochrome<sup>®</sup> Black T correctly indicated whether EDTA was present or not, in combination with  $\geq 1\mu$ g of calcium and  $\geq 0.1\mu$ g of magnesium, as long as the EDTA:ion ratio was  $\geq 3$  for calcium and  $\geq 10$  for magnesium. Normally occurring EDTA to calcium or magnesium ratios well exceed these values. Furthermore, resulting UV/Vis spectra were different between samples that tested positive and negative for EDTA using Eriochrome<sup>®</sup> Black T. Positive samples (blue) yielded a peak around 200nm; all other regions of these spectra exhibited negligible absorbance. Negative samples (pink) exhibited a weak, broad peak from 500nm–700nm, with a point of inflection around 590nm. Unfortunately, robust baselines could not be established at any of these regions, thereby preventing the formation of reliable conclusions. Last, testing with ATR/FTIR and Raman spectroscopy proved challenging with the small sample sizes tested and need additional troubleshooting.

Following proof of concept, various areas were explored to adapt the Eriochrome<sup>®</sup> Black T indicator for use with forensically relevant blood samples. Sample size was a significant concern and quickly led to the need to remedy color interference from blood itself, even from dilutions as low as 1:400. Several products or reagents were evaluated: Microcon<sup>®</sup> filters, dryer sheets, HemogloBind<sup>™</sup>, sodium hydroxide, ethanol, and amidine latex beads. Though many of them proved successful at removing the interfering red color from blood, nearly all adversely interfered with the indicator reaction and resulted in either false positives or negatives. Unexpectedly, performing a 96% ethanol wash was effective and yielded the expected results; this process will be pursued further. Transitioning from liquid blood to dried stains was also problematic anytime a cellulose-based material was used (specifically, cotton swabs and cotton T-shirts); use of foam swabs remedied this issue.

These methods show varying degrees of promise for the development of a robust EDTA detection process. Future work will aim to further optimize and validate suitable methods.

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## EDTA, Blood, Planted Evidence



### **B155 A Longevity Study on the Analysis of Sexual Lubricants When Stored in Different Environmental Conditions**

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**Learning Overview:** After attending this presentation, attendees will understand the effects of storage conditions on the analysis of sexual lubricants through Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART<sup>®</sup>-HRMS), Gas Chromatography/Mass Spectrometry (GC/MS), and Fourier Transform Infrared Spectroscopy (FTIR).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information and preliminary patterns of the longevity of sexual lubricants when stored in sexual assault kits within different storage conditions over time. This may aid in providing knowledge of the viability of trace evidence (e.g., sexual lubricants) when analyzed at different time periods post-collection.

Unfortunately, sexual assaults are a reality in today's society. Due to the understanding that DNA from seminal fluid is one of the main forms of evidence being used to connect a suspect to a victim or crime scene, there has been an increase in the use of condoms in sexual assault cases. This dilemma has led the community to research other types of trace evidence (e.g., sexual lubricants). Previous research performed at the National Center for Forensic Science has led to the development of the Sexual Lubricant Database that aids in identification of discernable groups within lubricant manufacturing types, as well as the ability to separate lubricants from personal hygiene products. The classification and characterization of lubricants is a relatively new approach in analyzing trace lubricant residue in an operational setting.

Current research is being performed to determine the length and optimal conditions appropriate for storing trace lubricant residue before the samples begin to degrade and pattern becomes unrecognizable. Prior to conducting the study, it was necessary to determine how long the storage study samples could be stored in the event that the samples cannot be analyzed on the day of collection. Therefore, a two-week study was first performed to determine the longevity of extracted lubricants when stored within solvents at -20°C. Second, a preliminary cotton swab optimization study was completed to determine which swab yielded the most effective lubricant extraction and least background noise. After these studies were conducted, sexual lubricant from a condom and a personal bottled lubricant were deposited onto respective cotton swabs to be stored and evaluated under different conditions.

This research project used previously developed DART<sup>®</sup>-HRMS, GC/MS, and FTIR protocols to analyze the stored swabs containing sexual lubricants at time points: 0 hours, 24 hours, 7 days, and 1 month. At each time point, a blank swab, a personal lubricant swab, and a condom swab were stored in different storage conditions. These storage conditions were 35°C to simulate a hot environment, 22°C at approximately 100% humidity, 22°C at approximately 50% humidity, 22°C at approximately 0% humidity, 4°C to mimic samples stored within a refrigerator, and -20°C to imitate samples stored within a freezer. Storage of swabs at these conditions allowed for the determination of lubricant degraded and adversely affected the ability to recognize classification patterns at the simulated environmental conditions. Additionally, this study permitted the determination of optimal storage conditions for swabs containing trace lubricant evidence from sexual assault kits. The blank swab, personal lubricant swab, and condom swab were extracted into methanol and were analyzed in triplicate via the DART<sup>®</sup>-HRMS, as well as FTIR, and in duplicate by GC/MS. The resulting spectra from each instrument can be used to classify unknown lubricant samples and generate investigative leads. Multivariate statistical techniques were used to compare the stored samples to the neat samples to determine if chemical breakdown for each lubricant occurred and if the pattern changed significantly. Preliminary results suggest that the general profile of the lubricant does not change; however, the concentrations of the components may decrease over time.

Analysis of stored sexual lubricants within different environments over time via DART<sup>®</sup>-HRMS, GC/MS, and FTIR may be beneficial to the forensic community by allowing investigators and forensic examiners to identify the optimal condition for storage to prevent the degradation of lubricants when samples cannot be immediately analyzed. Additionally, the ability to understand the effects of environment and time to the analysis of sexual lubricants may offer helpful information when analyzing sexual assault kits and provide a link for investigators between a suspect and crime scene.

#### **Sexual Lubricants, Longevity Study, Mass Spectrometry**



### B156 Forensic Education Needs for Local and Federal Hiring Managers From Job Applicants

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**Learning Overview:** The goal of this presentation is to educate academicians who teach in forensic programs what the needs are for the students they produce. Given the abundance of educational options available to students, guidance on what hiring managers are looking for is imperative for successful outcomes for both the applicant and the hiring laboratory.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing professors and students interested in forensic careers what skills will be necessary to hone in order to be successfully hired into a forensic laboratory and by providing data to inform the discussion surrounding what the current and future needs of local and federal employers really are, not what they are presumed to be, and to provide real examples of stellar and sub-par applications.

Forensic education programs have burgeoned in the past two decades. Some programs follow guidelines from Forensic Science Education Programs Accreditation Commission (FEPAC). There are also vast pools of educational institutions that adequately educate their students in fundamental sciences, such as chemistry, biology, and physics. Emphasis on specialization at colleges and universities has led institutions to tout more than 100 majors from which to choose.<sup>1</sup> Some newer majors have produced students with solid fundamental science grounding, but have left hiring managers with difficulties in assessing the adequacy of their skill set. Examples of degree programs that may cause forensic hiring managers to pause are majors such as “Applied Chemistry,” “Biotechnology,” “Environmental Toxicology,” “Pharmaceutical Chemistry,” and “Forensic Science,” to name a few. Degree programs often offer internships with real-world lab experience; the degree of relevancy to a forensic laboratory may only be explored during interviews or during a probation period.

In this joint session, representatives from different forensic science educational programs, local and federal hiring managers, as well as representatives of the legal profession and those who educate jurists will provide information and data to fuel discussion of “Are forensic science programs meeting the current and future needs of employers and the criminal justice system?” In this presentation specifically, hiring managers will discuss applicant pools for recent hiring cycles, the number of positions available, and the number of applicants and the number of individuals hired into the laboratory.

Examples from a local and federal laboratory will be thoroughly presented. As an example of job requirements, all entry-level and journey-level criminalists at one local laboratory are required to have graduated “from an accredited college or university with a bachelor’s degree in Criminalistics or a closely related physical or natural science which must include the successful completion of a minimum of seventeen semester (or equivalent quarter) units of chemistry course work. Chemistry coursework must include laboratory and cover general chemistry and organic chemistry.”

Many of the forensic disciplines have specific education requirements. These requirements can be found in various scientific working group recommendations and forensic standards. Applicants need to be cognizant of these requirements to even be considered for positions. Hiring managers will discuss these various specific requirements and examples will be provided.

To fill one vacancy for a journey-level Criminalist II DNA position at a local laboratory, a recruitment produced 17 applications, 14 of which made it from Human Resources (HR) to the laboratory; when reviewing transcripts and experience only 8 were determined to meet minimum qualifications. A separate Criminalist II firearms recruitment to fill four vacancies produced too few candidates at the Criminalist II experience level, thus an entry-level Criminalist I recruitment will be considered.

As another example, a federal laboratory system had 30 forensic chemist vacancies to fill. There were a total of 434 applicants. Of those, only 100 were deemed qualified by HR. After the interview and testing phase, only 15 applicants were chosen.

The education does not stop after making it beyond the interview. A clean background check is required. Accreditation agencies have requirements obliging laboratories to train their scientists on the job to learn the skills and methods in the laboratory. Accreditation requirements state that court testimony training, testing, and monitoring shall occur, emphasizing the important nexus between forensic science and the legal system.<sup>2</sup> Additionally, forensic standards exist that provide specific requirements for forensic science practitioner training, continuing education, and professional development (e.g., American Society for Testing and Materials [ASTM] E2917). Hiring managers will describe these standards and provide examples.

In order to span the gap between what forensic programs produce, what hiring managers require, and the knowledge scientists must have of courtroom procedures, dialog is imperative so that institutions are aware of the gaps that exist and to appropriately course-correct to produce employable and articulate forensic scientists.

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#### Forensic Education, Hiring Requirements, Forensic Skills



### **B157 Expert Forensic Testimony Training for Non-Analysts**

*David Jackson, MSc\*, Washoe County Sheriff's Office, Reno, NV 89512*

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**Learning Overview:** The goal of this presentation is to inform attendees about non-conventional training opportunities and methods to best serve the criminal justice system and to provide details of such opportunities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enthusing, educating, and informing attendees in the importance of delivering forensic testimony training to a wide range of end-users and through diverse methods.

This presentation regards the forensic scientist's responsibility to be equipped and seek opportunities to adequately and appropriately inform and train all members of the criminal justice system, especially those who have a vested interest in the expert witnesses testimony. During the course of a forensic scientist's training program, which can often be very lengthy, much emphasis is rightly given over to the practical aspects of bench work, data interpretation, report writing, and review.

While the aforementioned topics are extremely necessary portions of the training program, this presentation will encompass the opinion that an area of training that could be much improved upon concerns expert witness testimony. Not only are the testifying scientists in need of robust and realistic training for such an occasion that can often be out of their comfort zone, but so too are all other end users of the forensic testimony to be given before the court. Often neglected within training programs are the needs of all people within the courtroom and justice system to understand the intent behind the presentation of potentially complex results, interpretations, and opinions being offered by the highly skilled and technically trained scientist. This presentation will address the shortfalls inherent when the forensic science provider conducts their own internal witness training program that, within government laboratories, is generally tailored to prosecution lines of questioning. This is especially important during a time when the recent emphasis on attempting to minimize bias within the field is at the forefront of forensic conversation.

A universal training or teaching course is inadequate to fulfill the very different needs of the analyst, Law Enforcement Agency (LEA) officer, prosecution attorney, defense attorney, judge, and the general public (jury pool). The analyst may well be among the best equipped to assist the understanding of each of these contributors to justice, and this presentation will give examples and suggestions of how a crucial understanding may be instilled upon all stakeholders with clarity, succinctness, and even a touch of levity.

A unique position within the courtroom is held by the judge whose role within that arena and the judicial system is critical to the fair outcome of any legal hearing or trial proceeding. With such a burden comes the expectation by the court for a judge to make well-informed and just decisions during the admission of testimony. This presentation will additionally focus on the opportunities that exist for laboratory personnel to input in the continuous education of judges. Participation at this level of education is extremely beneficial to the individual judges, scientists, and the wider justice system. However, this opportunity is seldom taken; therefore, much encouragement will be given during this presentation toward greater involvement at this level.

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### **Court Testimony, Education, Training**





### **B158 Crime Scene Transformation: Helping Evidence Tell Its Story**

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**Learning Overview:** After attending this presentation, attendees will have learned how to transition a crime scene unit from a classified operation to a civilian unit and the downstream benefits to the crime laboratory's forensic analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a rubric for how to make the transition to and understand what efforts best serve the community, police department, inter-agency relationships, and other stakeholders. The discussion will include information about how the concept of a civilian and independent crime scene unit began, the efforts the City of Houston, the Houston Police Department, and other key stakeholders employed to make it a reality, and the belief that crime scene investigation is the face of forensics in the field and at the core of successful subsequent testing. The Houston Forensic Science Center (HFSC) has been nationally recognized for innovation and transparency in other forensic areas, but the crime scene unit's transformation has gone largely unnoticed. This presentation is aimed at sharing that unit's success and at educating others about the pros and cons of this transition.

Crime scene investigators are often those among the first, if not the first, to encounter evidence and the scene. As such, a unit's standard operating procedures impact not only what is identified as evidence, but how those items are collected, the level of potential contamination and/or loss, and whether the evidence is collected, preserved, handled, packaged, and identified in a manner that can potentially be used to further a criminal investigation. It is also where forensics comes into the most direct contact with law enforcement at a time when emotions are highest, a crime scene. As a result, transitioning from a classified to a civilian crime scene unit comes with trials and tribulations, and all aspects and phases must be considered, from the slow introduction of civilians into a unit that still has classified personnel, transferring those officers back to their law enforcement duties, and continuing to offer quality work while overcoming the obstacles.

The HFSC navigated all of this and more as it transitioned to a civilian crime scene unit. HFSC's successes, failures, and numerous challenges can now serve as an educational tool for others in the forensic community looking for ways to become more efficient and offer higher quality services to stakeholders. HFSC's Crime Scene Unit (CSU), like all other forensic disciplines in the agency, is now accredited by the American National Standards Institute (ANSI) National Accreditation Board (ANAB) to International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025 standard. This unit has changed the perception of Crime Scene Investigators (CSIs) from mere "technicians" or "garbage collectors," as they were called before HFSC took over, to colleagues with skills and critical thought processes that are crucial to an investigation.

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### **Cognitive Bias, Crime Scene Investigation, Evidence Handling**



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### **B159 Are Forensic Science Programs Meeting the Current and Future Needs of Prospective Employers?**

*Ruth Waddell Smith, PhD\*, Michigan State University, East Lansing, MI 48824; Glen P. Jackson, PhD\*, West Virginia University, Morgantown, WV 26506-6121*

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**Learning Overview:** After attending this presentation, attendees will be familiar with forensic science educational programs in the United States, the skill sets gained by students in these programs, and the subsequent placement of graduates into forensic science laboratories.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing data to inform the discussion surrounding forensic science educational programs meeting the current and future needs of employers.

Over the past decade, there has been an increase in the number of forensic science educational programs offered in the United States at both the undergraduate and graduate levels. In the early 2000s, the American Academy of Forensic Sciences established the Forensic Science Education Programs Accreditation Commission (FEPAC), with the aim of developing and implementing education standards to ensure the high quality of forensic science education programs. The FEPAC standards stipulate that forensic science degree programs must include core courses in forensic science topics and specialized science topics, as well as a capstone/research experience, which should culminate in a scholarly product, such as a presentation or publication. Further, programs must demonstrate interactions with forensic science laboratories that may include internships and collaborative research opportunities. Accreditation is granted for five-year cycles, and programs must undergo a rigorous reassessment process to be granted reaccreditation. As of July 2019, there are more than 25 undergraduate and 20 graduate programs currently accredited, meaning that each program has demonstrated implementation of and compliance with the standards.

In this joint session, representatives from different forensic science educational programs, local and federal hiring managers, as well as representatives of the legal profession and those who educate jurists, will provide information and data to fuel discussion regarding the question “Are forensic science programs meeting the current and future needs of employers and the criminal justice system?” In this presentation specifically, ways in which various forensic science educational programs implement FEPAC accreditation standards at both the undergraduate and the graduate level will be discussed. In one example, recent program changes that have been made to enhance the skill set of students will be presented, including additional required courses, a more formal research proposal presentation, and the incorporation of professional development opportunities. This presentation will also provide data on job placement of recent graduates and lessons learned from post-graduation surveys. In addition to FEPAC-accredited BS and MS programs, this presentation will also discuss the recent creation of two PhD programs in forensic science in the United States. This aspect of the presentation will describe the motivations and considerations for the PhD programs, the requirements of the programs, and some lessons learned following the first few years of operation of each program. Throughout the presentation, specific examples will be given to contribute to the discussion of “Are we meeting the current and future needs of employers and the criminal justice system?”

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#### **Forensic Science Education, FEPAC, Employer Needs**



### B160 Ridge Drift: The Relevance of a Not-So-Rare Fingermark Aging Phenomenon

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**Learning Overview:** After attending this presentation, attendees will understand that ridge drift is not an uncommon event in processed latent fingermarks and will recognize the variables that correlate to higher incidences of this phenomenon.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the practical implications when detecting ridge drift in processed latent fingermarks. Knowledge of the variables that affect ridge drift will inform attendees as to best practices when processing, comparing, and identifying fingermark evidence to prevent ridge drift from contributing to false negative conclusions.

Latent fingermarks recovered from crime scenes are compared with reference fingerprints to provide circumstantial evidence of the presence of an individual at a location. A limitation of this source attribution approach is the quality and quantity of the friction ridge characteristics, which can contribute potential sources of error to a comparison analysis. Further, the timeframe from occurrence to recovery may account for degradation effects that preclude a true contemporaneous comparison.<sup>1</sup>

A previous study by De Alcaraz-Fossoul et al. described a phenomenon, referred to as fingermark ridge drift, which was characterized as a random modification of aged fingermark patterns at a ridge scale.<sup>2</sup> This event was proven to alter key elements used for identifications, such as the physical appearance of minutiae. Little is currently known of the underlying cause or factors contributing to the occurrence of ridge drift. Currently, the Organization of Scientific Area Committees (OSAC) Friction Ridge Subcommittee documents do not consider fingermark ridge drift in order to account for possible dissimilarities during a comparison process. As a result, an examiner who compares an aged latent fingermark to a reference print (i.e., inked or scanned) without considering ridge drift may erroneously report an inconclusive result where a positive identification may be justified.

The present study was designed to both replicate and expand the variables of the previous ridge drift study by including 672 fingermarks from male and female subjects of similar age; two substrates (tile and plastic); two distinct color powders (carbon black and titanium dioxide–white); three light conditions (direct natural light, shade, and darkness) indoors; and two secretion types (sebaceous- and eccrine-rich). Fingermarks were simultaneously deposited, aged over a period of 2 to 72 days, and powdered for visualization. All developed prints (aged) were compared relative to fresh (day 0) latents to locate ridge drift. For the purpose of the study, ridge drift is collectively defined as either true drift (attributed to ridge movement) or selective degradation (visual modification of ridge patterns). Each processed fingermark was independently examined by three people for the presence of ridge drift. A conservative examination approach was adopted in identifying incidences of drift in that unanimous agreement was required among all three examiners. The number of prints containing drifts was considered rather than the individual counts per print. An inconclusive result was reported when discrepancies occurred among examiners. Further, fingermark images of poor quality (due to degradation effects or artifact inclusions) were also reported as inconclusive results.

Ridge drift was observed in 42 of 672 prints, while 168 prints were reported as inconclusive results. While incidences of ridge drift were detected in multiple fingermark samples across all independent variables, statistical analysis using a multinomial logistics model showed that only powder type, secretion type, and the substrate indicated a significant correlation with increased incidences of ridge drift. There was no significant correlation in the incidence of ridge drift with respect to the subject, time since deposition, or the environmental lighting conditions used. The incidence of ridge drift was significantly higher when black powder was used on plastic. Of note, although the focus of the study was detection of ridge drift, the number of inconclusive samples reported was higher for prints processed with black powder and found to be statistically significant.

The average observation of ridge drift in approximately 6% of samples processed in this study supports that ridge drift is not a rare event, particularly in light of the conservative analysis approach implemented. This is of even greater concern if a fingerprint examiner follows the International Criminal Police Organization (INTERPOL) European Expert Group on Fingerprint Identification II (IEEGFI II) recommendation that single minutiae differences are significant to the conclusion of an identification. Logically, this approach will increase the number of false negative conclusions. Ridge drift can provide useful information that can help reduce misidentifications.

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#### Ridge Drift, Environmental Conditions, False Negative



## B161 Accelerated Development of Latent Prints on Thermal Papers

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**Learning Overview:** The goal of this presentation is to demonstrate how heat and humidity can be judiciously applied to accelerate the development of latent prints on thermal papers without sacrificing print quality.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by debunking commonly practiced traditions that any application of heat or humidity will negatively affect thermal papers and thus enable forensic examiners to more expeditiously develop latent prints.

Latent print development on thermal paper is a time-consuming task for latent print examiners. Development methods applied to non-thermal papers have historically been modified for thermal papers. The addition of heat on thermal papers has been widely discouraged because the dyes in the active layer of thermal paper (commonly leuco dyes) change color in the presence of heat, acids, and electron-accepting compounds.<sup>1</sup>

Generally, latent prints on thermal papers are developed over 24 hours in the dark under laboratory environmental conditions.<sup>2-4</sup> However, some research has promoted the use of heat to preferentially develop a latent print on thermal paper using various tools, including a hairdryer, a metal press called the Hot Print System, and heated solid chemicals (i.e., 1,2-indanedione and zinc-chloride).<sup>5,6</sup> Consequently, it may be concluded that heat above ambient laboratory temperatures can aid in developing latent prints on thermal papers.

The goal of this work was to determine whether the use of heat and humidity via a humidity chamber can be used to accelerate the development of latent prints on thermal papers similar to how non-thermal papers are treated. The application of heat and humidity was evaluated for the accelerated development of prints using common porous substrate staining solutions, including 1,2-indanedione, ninhydrin, and 2-isononylninhydrin (i.e., Thermanin).

Fingerprints from multiple donors ( $n=20$ ) on a comprehensive range of thermal papers ( $n=13$ ) were developed using 1,2-indanedione, ninhydrin, and Thermanin. The quality of prints were measured using Universal Latent Workstation's (ULW's) LQ Metrics overall clarity score as well as scoring by five trained latent print examiners using unenhanced grayscale images. It was determined that heat (50°C) and humidity (60%RH) could be applied to thermal paper without inducing discoloration. Additionally, the prints developed using the aforementioned accelerated method (20min) were generally equal to or higher in quality than those produced over 24hrs under ambient conditions. These same findings held true for prints aged and tested out to 30 days post-deposition. Additionally, a thermal probe was developed to more accurately categorize the thermal sensitivity of thermal papers and thus screen new evidence for method compatibility. In summary, this technique successfully accelerated the development of latent prints on thermal papers from 24hrs to 20mins, thereby expediting evidence processing for any of the three processes without compromising the overall quality of the developed prints.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army (DA) or the Department of Defense (DoD). Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors, Defense Forensic Science Center (DFSC), United States Army Criminal Investigation Command, Office of the Provost Marshal General (OPMG), DA, or DoD.

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### Thermal Papers, Latent Prints, Heat and Humidity

## B162 A Microfluidic Device for the Identification of Biological Sex by Analysis of Fingermarks

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**Learning Overview:** As a result of attending this presentation, attendees will have introductory knowledge about a novel microfluidic technique for colorimetric determination of biological sex from the biochemical analysis of fingermarks.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a non-enzymatic, cost-efficient, microfluidic colorimetric assay that was developed to measure the arginine content in fingermarks. Rapid and objective determination of the biological sex of unknown individuals was accomplished using inexpensive instrumentation. Notably, the assay described herein is unaffected by the presence of magnetic, dual-use powder; this compatibility is imperative as this powder is commonly used to visualize latent fingermarks at a crime scene prior to further analysis.

Current fingerprint analysis techniques are largely visual and exclusionary, and therefore inherently require a reference for comparison. However, biochemical analysis of fingermarks is becoming more common and provides the opportunity to obtain unique donor identifiers without comparison to a reference. Fingermarks contain metabolites from several hormone-regulated metabolic mechanisms, which can serve as biomarkers or indicators of physiological attributes of the donor, such as age, ethnicity, health status, and gender.<sup>1-3</sup> For instance, in most cases, amino acid concentrations in fingermarks from a female donor are approximately two-fold higher than those deposited by a male.<sup>1</sup> Determination of amino acid concentration in fingermark deposits can thus be used to indicate the biological sex of the donor.

To date, most research regarding amino acid detection and quantification in fingermarks relies on spectrometric methods, including Raman and infrared spectroscopy. A significant disadvantage of such methods is the requirement for the use of sophisticated and expensive instrumentation by trained personnel. Consequently, on-site, chemical analysis of fingermarks is not commonly implemented. Notwithstanding, Brunelle et al. report a colorimetric method for the determination of the biological sex of a fingermark originator using Ultraviolet/Visible (UV/Vis) spectroscopy.<sup>4</sup> Though this represents progress toward minimizing assay cost, the instrumentation required is still quite expensive and necessitates non-trivial user training. Consequently, there is still a need for a rapid, inexpensive, and automated method of analysis that diminishes the need for significant user training for on-site use. Biometric data obtained onsite would facilitate accelerated determination and/or elimination of suspects, thereby increasing the efficiency of time-sensitive investigations.

In this research, the Sakaguchi colorimetric test was adapted to a rotationally driven microfluidic platform and used to detect and quantify arginine in fingermarks deposited by male and female donors. Observation of a red color following the reaction is indicative of the presence of arginine in a given sample. The intensity of this color is linearly proportional to the concentration of arginine in fingermark samples. Objective detection and quantification of arginine was accomplished using open-access image analysis software based on this colorimetric result. The mean concentration obtained experimentally for samples from female donors, 79.60 $\mu$ M, was not statistically different from the theoretical value, 94.8 $\mu$ M ( $p=0.2562$ ).<sup>5</sup> For males, the experimental value obtained was 42.2 $\mu$ M, which was not statistically different from the theoretical value, 54.0 $\mu$ M ( $p=0.3666$ ).<sup>5</sup> Conversely, the experimental means from male and female samples were found to be statistically different from each other with  $p < 0.0001$ . This indicates that the microfluidic protocol could reliably be used to differentiate between male and female fingermarks.

Following initial chemical optimization, compatibility of the assay with magnetic, dual-use powder was evaluated by including a powder-dusting step prior to further analysis. The application of magnetic powder was included as a means of approximating “real world” conditions at a standard crime scene. The magnetic particles were removed following hydrolysis of the fingermark samples. It was observed that despite the inclusion of the magnetic powder, minor modifications of experimental conditions resulted in the expected development of color and intensity.

The novel microfluidic assay for determination of biological sex from fingermark deposits described herein can not only be used to reproducibly differentiate between male and female donors, but is also portable and automatable, making it conducive to use onsite by untrained personnel.

### Reference(s):

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### Microfluidics, Fingermarks, Colorimetric Analysis

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### B163 Analyzing Latent Print Chemistry Using 2D Gas Chromatography

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**Learning Overview:** After attending this presentation, attendees will have learned of another way of analyzing latent prints based on the chemistry in addition to evaluating the quality of the print and how different correlation statistical methods, such as Pearson's correlation, aids in inter- and intra-variability based on the participant's chemical composition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a new instrumental technique in analyzing latent print chemistry that would be a supplement to traditional latent print examination.

Latent print evidence when received from a crime scene are analyzed and compared by examiners using the Analysis, Comparison, Evaluation-Verification (ACE-V) protocol. This accounts for the analysis of the questioned print using Type 1, 2, and 3 minutia details, comparing it to a known exemplar print either obtained from the Automated Fingerprint Identification System (AFIS) or any potential suspects. However, there may be benefit to the community to evaluate the chemistry of the latent print in addition to the analysis of physical minutiae because the chemistry of the naturally secreted sweat and oils is relatively consistent, aside from illnesses and the use of personal hygiene products (e.g., lotions, hair oils, etc.).

Over the years, researchers have evaluated the use of various instrumental techniques in analyzing latent prints, such as Gas Chromatography/Mass Spectrometry (GC/MS), matrix-assisted laser desorption/ionization, and Raman and infrared spectroscopy. GC/MS has been used in latent print studies in identifying lipids and amino acids observed in different volunteer groups based on age, gender, and ethnicity, along with determining the age of the print from deposition to collection; however, there are areas where co-eluting compounds were present within the chromatograms that could decrease the potential of accurate associations or discrimination between unknown and known comparison latent prints.

In this research, 2D GC/MS was used to determine if latent prints could be linked to a source. Statistical techniques, such as Pearson's correlation coefficient, was used to determine the inter- and intra-correlation values between latent prints deposited by various volunteers. In a second study, it was determined latent prints could be classified into either age, gender, or race/ethnicity groups based on chemical composition. Latent print samples collected from volunteers were deposited onto a microscope slide and a microfiber filter, which were extracted using dichloromethane or hexane, and evaporated under nitrogen. To afford for better chromatographic separation and detection along with decreasing volatility, the samples were derivatized before GC analysis. Boron Trifluoride in Methanol (BF<sub>3</sub>-MeOH) and n-Methyl-n-(trimethylsilyl)Trifluoroacetamide (MSTFA) was used for sample derivatization, and the reagent that displayed the better peak identification based on percent quality match, abundance, and reproducibility was used for the duration of the study.

The results of the latent print samples were subjected to compound identification and compared visually for similar/dissimilar peaks between volunteers. The latent print samples were either linked back to a source using Pearson's correlation values or categorized into a class (i.e., race, gender, or race/ethnicity) using principal component analysis and k-nearest neighbors. Error rates were determined to test the validity of the statistical methods described for the samples obtained from the 2D GC/MS.

#### Latent Print Chemistry, 2D GC/MS, Association Error Rates



### B164 Lessons Learned From Conducting Black-Box Evaluations in Multiple Disciplines

R. Austin Hicklin, PhD\*, Noblis, Reston, VA 20191; JoAnn Buscaglia, PhD\*, FBI Laboratory, Counterterrorism and Forensic Science Research Unit, Quantico, VA 22135

**Learning Overview:** The goal of this presentation is to help attendees understand the issues involved in designing and conducting black-box evaluations of the accuracy and reliability of forensic examiners, across several forensic disciplines.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing approaches to assess potential areas of strength and weakness in multiple areas of forensic science.

**Hypothesis Statement:** Lessons learned from black-box evaluations in a forensic discipline can identify issues for consideration in the design of studies assessing the accuracy and reliability of examiners in other forensic disciplines.

The 2016 President's Council of Advisors on Science and Technology (PCAST) Report on forensic science stated that black-box evaluations are necessary to establish the validity of forensic examination methods that rely on human judgment and provided some guidance on what criteria PCAST required for such testing to be considered rigorous and suitable for court admissibility.<sup>1</sup> The accuracy, reproducibility, and repeatability of conclusions in a number of forensic science disciplines have not yet been assessed using such black-box studies. The results of such studies would assist in supporting the scientific basis of these forensic disciplines and provide insights into improved operational procedures and training.

The forensic science community is currently engaged in a variety of efforts to evaluate the accuracy and reliability of forensic conclusions in the pattern evidence disciplines. This presentation will discuss the issues involved and lessons learned from designing and conducting black-box evaluations in five forensic disciplines, including completed studies of latent print examination and studies in progress of handwriting examination, footwear examination, bullet examination, and bloodstain pattern analysis. This presentation will discuss what aspects of study design and data collection have commonalities that apply to multiple disciplines and the aspects that are specific to individual disciplines.

This presentation will discuss topics that should be considered in the design of black-box evaluations, including: (1) controlled collection of samples to simulate operational casework; (2) accounting for different conclusion scales; (3) quality assurance; (4) analysis methods; (5) distribution of physical vs. electronic samples; and (6) evaluation based on classification of individual samples (e.g., bloodstain pattern analysis) vs. comparison of multiple samples.

These studies are designed to evaluate and/or demonstrate the validity of each discipline, as well as providing essential information to laboratory managers and practitioners that may be used to improve or enhance practices in the laboratory. This presentation will also discuss how the results of black-box studies provide data for subsequent "white-box" evaluations and present lessons learned from designing and conducting white-box testing.

#### Reference(s):

<sup>1</sup>. Executive Office of the President, President's Council of Advisors on Science and Technology (2016) Report to the President. *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*. September 2016.

#### Evaluation, Error Rates, Examiner Accuracy



### **B165 Using a Smart Phone Special Gadget to Find and Collect Latent Fingerprints: Simplifying the Process**

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**Learning Overview:** After attending this presentation, attendees will have learned about an innovative use of a smart phone, equipped with a special and simple gadget, to acquire latent fingerprints in crime scenes with a low-cost, non-destructive method.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the possibility of using a smart phone, with a low-cost gadget and without any chemical substances, to highlight and acquire latent fingerprints in crime scenes, improving the productivity and cost of forensic examination, especially in developing countries.

Latent digital prints are important vestiges in crime sites, due to their high incidence and possibility of author identification. However, their acquisition usually depends on the personal experience of the forensic examiner regarding knowledge of classical methodologies and the correct use of reagents.

Although there are new technological tools commercially available for use in the search and acquisition of fragments, such resources are still restrictive to Brazilian professionals because of their high cost. As a consequence, the registration of papillary fragments ends up depending on classical methods, which involves the use of chemical reagents, causing modifications that destroy the vestige as originally conceived. Most of these chemical reagents also have high toxicity, which can impair the health of the examiners.

This research proposes the development of a simple and low-cost device, designed to adapt to a smart phone, allowing the enhancement of latent papillary fragments and their capture through the smart phone camera using optical methods. The built prototype is simple, lightweight, and enables the proposal of a non-destructive and low-cost methodology, without any chemical interaction with the evidence, preserving the vestige as originally conceived, and allowing its use in other methodologies, such as spectrophotometry and touch DNA.

The prototype was tested with a medium technology smart phone in laboratory and under external conditions. Latent fingerprints were acquired from non-porous surfaces, frequent in crime scenes, such as glass, metal, and plastic. Tests were also performed focusing on other possible applications of the method, such as using mass and oil to analyze the results of modeled and stamped fingerprints.

The results were confronted with known fragments, and the comparisons demonstrated sufficient quality for at least 12 matching points (Brazilian standards). Both the device and the developed methodology showed an excellent performance for the acquisition of latent papillary fragments on different surfaces and conditions. The results obtained with the gadget allows a new step for the research, which would be tests in crime scenes.

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#### **Fingerprint, Dactyloscopy, Non-Destructive**





### **B166 A Cyanoacrylate Chamber for Vehicles: Innovation and Astonishing Results in Brazilian Casuistry**

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**Learning Overview:** The goal of this presentation is to show the possibility of increasing the number and quality of developed latent fingermarks in vehicles with the use of a cyanoacrylate chamber for vehicles, especially when the powder method would exhibit limitations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining an exclusive project developed by fingerprint experts from Brazil, the cyanoacrylate fuming chamber for vehicles, that is fully automated, which guarantees scalability in the amount of material to be processed and safety for the experts and to the environment. This processing allows revealing latent fingermarks in a high-quality standard and on uncommon surfaces when compared to other conventional methods.

The analysis of latent fingermarks stands at the forefront of criminal investigations, especially in crime scene casework. Their detection on smooth surfaces, like metals, plastics, or glass by Cyanoacrylate Ester (CA) fuming is a commonly used technique worldwide.<sup>1</sup> In fuming chambers, the CA is vaporized and polymerizes as a white solid residue on the surfaces of the potentially manipulated objects. In Brasília, Brazil, vehicles commonly used to be analyzed by visual inspection and treated with the black fingerprint powder/lift technique. Considering that the CA method is capable of enhancing the quantity of ridge detail as well as better quality fingermarks being developed when compared to the powder methods, fingerprint experts from Brasília projected, installed, and validated a CA Chamber for Vehicles (CCV), which is considered the largest in the world (6.2×4.0×3.1m). This was the result of an exclusive project developed by fingerprint experts that work in the Laboratory of Fingerprint Enhancement Methods of the Civil Police of the Federal District. Usually, the CVV is used to process vehicles related to homicides, large thefts, and robberies and allows processing a vehicle as large as a pickup truck. There is an internal division isolating one-third of its volume for daily use to process small- and medium-sized objects recovered from crime scenes and two-thirds of its volume is designated to the vehicle. The operating cycle was tested and automated in three steps: (1) pre-humidification to about 80% by water evaporation using a boiler; (2) CA fuming using 16 electric heaters (180–200°C) distributed inside and outside the vehicle for 40min; and (3) CA fume exhaustion for 40min. The equipment also includes an air circulation device to homogenize humidity and a CA fume inside the chamber.

Vehicle processing in CCV enables visualization of fingermarks on surfaces that would rarely be appropriate for the application of other techniques. Positive results were obtained on the turn signal, windshield wiper handle, central panel, glove compartment, driver's seat regulation handle, emergency brake button, ignition, steering wheel, motor compartment, gear shift, headrest stand, inside door handle, and seatbelt buckle. Since the inauguration of CVV (approximately ten months), 26 vehicles have been processed and 17 suspects were identified based on the fingermarks developed on vehicle structures. Six of these suspects have previously been criminally identified and one of them was identified in two different vehicles. For vehicles treated by the black powder method and subsequently processed in the CVV, an increase in the number of developed fingermarks was observed. In addition to an increase in the number, the new equipment allowed an increase in the quality of fingerprint evidence, especially when the powder method would show limitations. The acquisition of CVV represented a significant improvement in the expert routine of the Identification Institute of the Civil Police of the Federal District and should be encouraged for other forensic laboratories around the world. The results have helped to solve several criminal cases in the Federal District.

#### **Reference(s):**

<sup>1</sup>. F.G. Kendal. Super glue fuming for the development of latent fingerprints. *Identification News* 27 (5) (1982) 3-5.

#### **Cyanoacrylate Chamber, Vehicles, Brazil**



### B167 Fingerprints and Ancestry: Is It in the Details?

Jessica R. Ford\*, Oklahoma City Police Department, Oklahoma City, OK 73135

**Learning Overview:** This presentation will discuss the plausibility of using Galton features, specifically the certain types of Galton features to determine a person's ancestry.<sup>1</sup> Such research could aid law enforcement in shortening an otherwise lengthy suspect list or even aid in the exoneration of those wrongfully convicted.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by creating a greater understanding of criminalistics, specifically as it relates to the viability of fingerprints left at a crime scene and new tools that could be used to determine a fingerprint's origin.

Over the years, Sir Francis Galton, and more recently, Fournier and Ross have explored this concept of ancestry determined from fingerprint minutiae.<sup>1,2</sup> Galton studied and compared the fingerprints of English pure Welsh, Hebrew, Black, and some Basques from Cambo in the French Pyrenees to look for differences in patterns and minutiae characteristics.<sup>1</sup> His study had a sample size of more than 100 individuals. While his study did not yield significant scientific results, Galton claimed to be able to tell a difference in fingerprint minutiae between the different ancestries. Fournier and Ross conducted a similar study with the purpose of exploring the influence of sex, ancestry, and pattern type on minutiae in African descent and European descent males and females.<sup>2</sup> Overall, 243 right index fingerprints were chosen, including 61 African American females and 61 African American males for a total of 122 African Americans, as well as 60 European American males and 61 European American females for a total of 121 European Americans. Based on this study, fingerprint minutiae, specifically the total number of bifurcations, shows promise as a method to predict the ancestry of an individual to some degree of certainty.

Researchers at the University of Central Oklahoma conducted a study made up of 250 participants, 25 Hispanic-descendant males, 25 Hispanic-descendant females, 25 Asian-descendant males, 25 Asian-descendant females, 25 Native American-descendant males, 25 Native American-descendant females, 25 African-descendant males, 25 African-descendant females, 25 European-descendant males, and 25 European-descendant females. The sex and ancestry of each participant was ascertained based on self-identification and demographic information. In order to collect the prints, each participant had their right index finger rolled on an index card using fingerprint ink. Each print shows a complete nail-to-nail roll recorded for later analysis of friction ridge detail. Because of the statistical likelihood that the right index finger is the print most often encountered at crime scenes, those prints were used for analysis. With assistance of AFIX Tracker® technology, each print was analyzed and marked by the researcher for each of the five main fingerprint minutiae characteristics: bifurcations, enclosures, dots, ending ridges, and short ridges. It has been found that the amount of bifurcations in one's fingerprint yields significant results as it relates to ancestry. Interestingly, it has been found that African American descendants have slightly more bifurcations than European American descendants.

Significant results in the above research highlights new possibilities for law enforcement personnel. Deriving someone's ancestry from their fingerprints can serve as corroborative evidence that could aid in the conviction of criminals who could otherwise walk free. Additionally, this forensic tool could also aid in the exoneration of those wrongfully convicted. This presentation could greatly impact the forensic science community by shedding light on innovative ways that fingerprints can be used in the application of criminalistics to crime scenes.

#### Reference(s):

1. Galton, S.F. *Finger prints*. London: Macmillan and Co. (1899).
2. Fournier, N. and Ross, A. Sex, Ancestral, and Pattern Type Variation of Fingerprint Minutiae: a Forensic Perspective on Anthropological Dermatoglyphics. *American Journal of Physical Anthropology*. (2015).

#### Fingerprints, Ancestry, Minutiae



### **B168 The Formation and Examination of Bloody Friction Ridge Patterns on Common Textile Materials Using Different Enhancement Techniques**

*Kaitlyn Chetney\*, Shorewood, IL 60404; Ralph R. Ristenbatt III, MS, Pennsylvania State University, University Park, PA 16802*

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**Learning Overview:** After attending this presentation, attendees will better understand which chemical and dye enhancement techniques can be used to enhance latent (blood) friction ridge patterns on various fabrics.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing criminalists with information as to which techniques are best suited for the enhancement of fingerprints composed of blood on fabrics that may be found at crime scenes.

The presence of fingerprints at a crime scene has proven to be useful during an investigation due to the great variability of ridge features. Finding and analyzing fingerprints may provide criminalists with information allowing an individual to be linked to an incident. It is common to find fingerprints, along with other friction ridge patterns, at crime scenes on a multitude of surfaces. For example, textiles of various types and complexity are often present at crime scenes. If a textile contains differentiating pieces of evidence, such as fingerprints composed of blood, it could be instrumental in the resolution of the investigation. However, bloody fingerprints on textiles may often be difficult to observe or locate, causing them to remain undetected or be deemed unsuitable for comparison. The enhancement of bloody fingerprints on fabrics could be crucial in increasing their probative value in an investigation.

There are several techniques that may be used to enhance fingerprints generated by blood-contaminated friction ridge skin. Criminalists must first characterize the fabric containing the pattern. Composition, structure, color, and textile porosity must be considered before selecting an appropriate chemical or dye. Three different classes of compounds that can be used to enhance fingerprints made primarily of blood are protein dyes, heme-reactive compounds, and amino-reactive compounds.

A series of five bloody fingerprints created with a blood-covered finger were deposited onto a variety of textiles. This was accomplished by depositing 20 $\mu$ L of defibrinated ovine blood onto the fingertip of the index finger (palmar surface of the distal phalange) and redistributing the blood with a Teflon<sup>®</sup> spatula, followed by placement of the fingertip onto the substrate. Four subsequent fingerprints were deposited onto the substrate without applying additional blood. The fingerprints were deposited onto both washed and unwashed fabric swatches of different colors with different backing materials.

The fabric swatches were allowed to dry for a specific amount of time (1 hour, 5 hours, 10 hours, or 24 hours), then fixed and/or enhanced using a variety of different dyes and chemicals (Amido black, Coomassie blue, Leucocrystal Violet, DFO, Hungarian Red, or Acid Yellow 7). After enhancement, a Nikon<sup>®</sup> D810 Digital Single-Lens Reflex (DSLR) camera was used to record the results of each trial. Different optical techniques were employed in an attempt to provide further enhancement.

The enhancement of patterns of friction ridge skin composed of blood on different fabrics may be important in forensic investigations. If better understood, criminalists may be able to decide which technique would provide optimal results for the enhancement patterns of blood on fabric.

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#### **Blood, Fingerprints, Enhancement**



## B169 Fingerprint Error Rate on Close Non-Matches (CNMs)

*Jonathan J. Koehler, PhD\*, Chicago, IL 60611; Shiquan Liu, PhD, China University of Political Science and Law, Beijing 100000, CHINA*

**Learning Overview:** The goal of this presentation is to provide attendees with information about the risk of false fingerprint identification in cases in which prints from different sources have many common features and few dissimilar features.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by making attendees aware of data related to how often expert fingerprint examiners err in difficult cases.

The accuracy of fingerprint analyses is critically important to the administration of criminal justice. However, a 2016 Report by The President's Council of Advisors on Science and Technology (PCAST) indicated that there were only two well-designed empirical studies that directly examined the accuracy of fingerprint examiners' identification conclusions.<sup>1</sup> These two studies reported very low false positive error rates on print pairs that the authors described as "challenging" and "representative of case work."<sup>2,3</sup>

Though encouraging, it is not clear whether similarly low error rates would be observed for CNMs in which the latent and exemplar prints have many common features and few dissimilar features. It is important to learn more about CNM error rates because CNMs are more likely to arise in casework as database searches become more common and as the size of the searched databases increases.

In the present study, high-level fingerprint examiners from 125 Chinese fingerprint agencies completed a mandatory online proficiency test using Picture Annotation Software 4 (PiAnoS4) fingerprint software that included two CNMs. PiAnoS4 enables participants to conduct a detailed, annotated, step-by-step examination of print pairs using the standard fingerprint method. Examiners viewed eight consistent points of comparison (minutiae) between the prints followed by an inconsistent point. Examiners were instructed to provide one of three conclusions for each latent-exemplar print pair: identification, exclusion, or inconclusive. In keeping with casework practices in China, the instructions discouraged reliance on the inconclusive conclusion. Examiners were also encouraged to provide written comments that document their thoughts at various decision points. Each agency received a unique user name and code. Agencies had five days to complete the test and submit results.

The false identification rates on the CNMs were 15.9% (17 out of 107, 95% Confidence Interval (CI): 9.5%, 24.2%) and 28.1% (27 out of 96, 95% CI: 19.4%, 38.2%), yielding a combined false identification rate of 21.7% (44 out of 203, 95% CI: 16.2%, 28.0%). These false positive error rates are higher than those observed by Ulery et al. and Pacheco et al., but they are roughly in line with error rates observed elsewhere on difficult CNMs.<sup>1,2</sup> Written comments from examiners who committed identification errors suggest that those examiners frequently failed to note any important points of difference between the latent and exemplar prints. A smaller percentage of examiners who committed identification errors observed dissimilarities, but they often rationalized them away as unimportant artifacts.

This presentation concludes that the false identification rate on CNMs may be substantially higher than the error rate identified in the two well-designed studies singled out in the PCAST Report. If so, then the probative value of reported identifications may be lower than widely believed in cases where the risk of encountering a CNM is amplified.

### Reference(s):

1. Ulery, Bradford T., Hicklin, R. Austin, Buscaglia, JoAnn., Roberts, Maria Antonia (2011). Accuracy and reliability of forensic latent fingerprint decisions. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7733-7738.
2. Pacheco, Igor, Cerchiai, Brian, Stoiloff, Stephanie. (2014). *Miami-Dade research study for the reliability of the ACE-V process: Accuracy & precision in latent fingerprint examinations.* (Document No 248534, Award Number 2010-DN-BX-K268).
3. President's Council of Advisors on Science & Technology, Executive Office of the President (2016). *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods.*

### Error Rate, Close Non-Match, Fingerprint



### B170 Testing the Accuracy and Reliability of Palmar Friction Ridge Comparisons: A Black Box Study

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**Learning Overview:** After attending this presentation, attendees will be aware of the results of a recent large-scale black box study that measured the performance of expert friction ridge examiners to establish a discipline error rate estimate for the comparison of palmar impressions. This is the first study to specifically measure performance on the palmar comparison task.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an error rate estimate that can be used by examiners in court when testifying to the results of palm comparisons. These results provide the first step in establishing the foundational validity of palmar comparisons, as defined by the recent President's Council of Advisors on Science and Technology (PCAST) Report.<sup>1</sup>

In 2011, a team of researchers from the Federal Bureau of Investigation (FBI) and Noblis published in the Proceedings of the National Academy of Sciences the first large-scale black box study measuring the accuracy of fingerprint examiners.<sup>2</sup> They reported a low rate of false positives (0.1%) and a rather high rate of false negatives (about 7.5%). The FBI/Noblis study dealt only with marks and prints originating from the distal phalanges of fingers (fingerprints). However, anecdotally it is estimated that approximately 30% of comparison cases involve palm impressions. It has been unknown up to now whether examiners are equally accurate at both tasks. This presentation provides the results of a recent large-scale black box study that measured examiners' accuracy when conducting exclusively palm comparisons.

This presentation reports on the results recorded both during the Analysis phase and the Comparison phase by a total of 226 fingerprint examiners who carried out a total of 12,279 determinations in Analysis and 9,460 decisions following Comparison. The pool of cases was composed of 526 cases (questioned and known palm impressions) of known ground truth (i.e., the source of the unknown impressions was known to the researchers before conducting the study). Both known mated pairs and known non-mated pairs were presented. Participants first performed a suitability analysis on unknown mark; thus, not all unknown marks proceeded to comparison (those deemed by the examiner to be unsuitable were not presented with a known exemplar to compare). Unknown marks and known exemplars varied in quantity and quality of features to reflect the complexity of casework.

Two online Shiny applications will also be presented for exploring the results of the study and the data's associated confidence and credible intervals. The implications of these results on the reporting of "error rates" associated with palm print examinations will be discussed along with the implications and incidence of "questionable" conclusions that may not be supported by a consensus panel.

#### Reference(s):

1. PCAST. *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*. Report to the President (2016), <https://obamawhitehouse.archives.gov/administration/eop/estp/pcast/docsreports/>.
2. Bradford Ulery, R. Austin Hicklin, JoAnn Buscaglia, and Maria Antonia Roberts. Accuracy and reliability of forensic latent fingerprint decisions. *PNAS* 108, no. 19 (May 2011): 7733-7738, <https://doi.org/10.1073/pnas.1018707108>.

#### Error Rate, Black Box, Fingerprints



### **B171 Unusual Fingerprint Patterns in a North Indian Population—Implications in Forensic Casework and Fingerprint Research**

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**Learning Overview:** After attending this presentation, attendees will understand the usefulness, importance, variability, and implications of the unusual fingerprint patterns in forensic casework and fingerprint research, especially while classifying the fingerprint patterns into defined categories.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community regarding new information on unusual fingerprint patterns. Moreover, this presentation is also expected to further help forensic scientists and fingerprint researchers acquire profound understanding of the fingerprint pattern in the identification process and in making population standards pertaining to different types of fingerprints in various population groups.

Fingerprinting remains one of the most popular methods of identification since the denouement of 19<sup>th</sup> century. Fingerprints serve as a powerful and most widely used characteristic tool in various issues pertaining to identification. The science of fingerprints has been employed for business transactions in Babylon up to the present day biometric, security system, and fingerprint sensing for locking of smart phones to secure the privacy of the user. The presence of definitive patterns and a large number of combinations of their occurrences form the basis of its use in forensics for identification purposes. The tenacity of fingerprint evidence for the identification of criminals has been well documented, but none of the research work focused on unusual fingerprints; therefore, the present study attempted to define such rarely encountered fingerprints in a population. The objective of the present study was to explore the unusual pattern types on the finger balls and their comparison with the commonly occurring and classifiable pattern types. Further, this presentation discusses the occurrence of unusual fingerprints in both the sexes with reference to the finger digits.

The research was conducted on 512 participants aged between 18 and 35 years from two ethnic groups residing in Districts Solan and Shimla in Himachal Pradesh State of North India. A total of 5,120 fingerprints from each digit of the 512 individuals were obtained. The fingerprints were analyzed for the pattern types based on Henry's classification. The patterns that did not fall in the prescribed category as per the definition of the finger pattern types were identified as unusual fingerprints.

A total of 11 unusual fingerprints were reported in both the sexes and two different ethnic groups studied, thus accounting for ~0.21% of the unusual fingerprints. These fingerprints were thoroughly analyzed and compared with the general pattern types according to Henry's classification. Furthermore, this study attempted to define these unusual fingerprints to formulate a category that may aid in the individualization process. The suggestive definition and naming of these new fingerprint patterns stands as a central pocket whorl (two patterns), a single spiral whorl enclosing mesh, a central pocket loop with a diminutive loop, and a lateral pocket loop with triradii (left) in the center. The proposed new nomenclature of these unusual fingerprints is expected to assist the forensic scientists and fingerprint examiners in better understanding and labeling of similar patterns in forensic cases.

#### **Criminalistics, Unusual Fingerprints, Fingerprint ID and Research**



### **B172 Do We Understand the Factors That Influence Fingermark Detection?**

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**Learning Overview:** After attending this presentation, attendees will have an improved understanding of the variety of factors that affect the detection of a latent fingermark. The goal of this presentation is to provide an up-to-date review of the factors influencing fingermark detection and how these can be determined and studied.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information to address a current knowledge gap and by encouraging the community to appreciate the complexity of this area. This presentation will: (1) assist researchers in fingermark detection to undertake more robust research; and (2) assist practitioners to be more proficient through a better understanding of the fundamentals of latent fingermarks when implementing detection methods in casework.

The primary aims of fingermark detection research are to improve the quality and number of recovered fingermarks. This is usually performed through the development of new methods and technologies to provide alternatives to or improve current procedures. While research of this nature is important to pursue, it fails to address the underlying question related to the factors that affect the detection of a latent fingermark. There has been significant research that has examined the differences between techniques, donors, and fingermark age, as well as the composition of latent fingermarks. However, previous research tends not to focus on determining how these factors influence the quality of the developed mark.

A previously published study found that substrate characteristics and fingermark donor inter- and intra-variability play major roles in the number and quality of marks developed.<sup>1</sup> Based on the findings of this initial study, there is still more research required to understand the fundamentals of latent fingermarks and their detection. This presentation will provide an update on the continuing investigations being performed to better understand the factors that influence fingermark detection, focusing on the second phase of the study using a larger donor population under more realistic conditions. The discussion will cover a number of related areas, including: (1) the influence of substrate chemical and physical characteristics on the development of fingermarks, and (2) the influence of the donor on the detection process, particularly cyanoacrylate fuming on plastic substrates. In both phases of the study, a greater donor dependency was observed for cyanoacrylate fuming than for indanedione-zinc.

It is argued that such research provides much-needed information to address the current knowledge gap. It also encourages the forensic science community to appreciate the complexity of this area. The findings from this study assist researchers in fingermark detection to undertake more robust research. For example, these findings provide guidance when developing experimental design by taking donor/fingermark composition and substrate characteristics into consideration, in particular for positive early studies. These findings also assist practitioners to be more proficient through better understanding the fundamentals of latent fingermark detection processes when implementing these methods in casework, including validation and refinement of Standard Operating Procedures (SOPs).

#### **Reference(s):**

- <sup>1</sup> Chadwick, S., Moret, S., Jayashanka, N., Lennard, C., Spindler, X., Roux, C. Investigation of some of the factors influencing fingermark detection. *Forensic Science International*, 2018, 289, 381-389.

#### **Fingerprints, Detection Techniques, Fingermark Compounds**



## B173 A Comparison of 2D Footwear Images Using Maximum Clique (MC) and Speeded-Up Robust Features (SURFs)

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**Learning Overview:** The goal of this presentation is to introduce an objective, accurate, and reliable approach to quantify the similarity between two outsole impressions.

**Impact on the Forensic Science Community:** Footwear impressions are commonly found in crime scenes, but examiners lack objective approaches to analyze and interpret the evidence. This presentation will impact the forensic science community by presenting an algorithm to quantify the similarity between two impressions and enable calculation of the probative value of footwear impressions in real casework that would have a big impact among footwear examiners.

Footwear examiners are tasked with determining whether the suspect's shoe could have left the print at the crime scene. Current practice relies on the visual comparison of the two impressions and a subjective assessment of their similarity, perhaps with the aid of a catalog of outsole patterns with brand and model information.

Footwear evidence are found in about a third of all crime scenes.<sup>1</sup> However, footwear impressions are rarely introduced as evidence in criminal proceedings. This may be because accurate, reliable, and validated methods to quantify the similarity between two outsole impressions have yet to be proposed, and therefore, examiners are limited in the type of conclusions they can make.

A new method called Maximum Clique, Comparison, Speeded-Up Robust Features (MC-COMP-SURF) that quantifies the similarity between two outsole impressions is proposed. The method compares a full or partial image of a shoe impression (Q) from an unknown source to an impression (K) from a known reference shoe, using 2D images of the impressions. MC-COMP-SURF relies on robust features on each impression and aligns them using MC.<sup>2</sup> MC arises from graph theory and can be used to assess the property of geometrical congruence in the outsole patterns. An advantage of the MC method is its invariance to rotation and translation. A disadvantage is that for large images, the method can be computationally intensive and time consuming. After aligning features in Q and K, it is possible to define multiple similarity features and combine them into a univariate similarity score using a Random Forest (RF). The approach that outputs an RF score from MC-COMP-SURF is denoted RF-SURF.

A large experimental database of 2D outsole images was built by researchers in the Center for Statistics and Applications in Forensic Evidence (CSAFE). One hundred sixty participants were allocated a new pair of athletic shoes of one of two brands and sizes 8, 8.5, 10, 10.5. Each shoe from each pair was imaged four times right after purchase and about every eight weeks on three additional occasions, during which time study participants wore the shoes. The algorithms discussed in this presentation were constructed and tested using a subset of the data consisting of shoes with the same class characteristics and similar degree of wear that are difficult to tell apart. RF-SURF outperformed other methods in the literature, including Phase-Only Correlation (POC) and MC-COMP-edge in the sense of minimizing classification error into the same or different shoe classes.<sup>3,4</sup>

The algorithm was also tested in more realistic scenarios with partial, degraded, and smudged images from the questioned impression. Images obtained from Q were degraded sequentially, resulting in images of decreasing clarity. The same algorithms were used to quantify the similarity between degraded images from Q and a high-quality image of K. Results suggested that the comparison algorithm based on SURF is robust to some forms of image degradation; at all levels of degradation, MC-COMP-SURF outperformed all other methods in terms of classification accuracy.

*This work was partially funded by the Center for Statistics and Applications in Forensic Evidence (CSAFE) through Cooperative Agreement 70NANB15H176 between the National Institute of Standards and Technology (NIST) and Iowa State University, which includes activities carried out at Carnegie Mellon University, University of California Irvine, and University of Virginia.*

### Reference(s):

1. William J. Bodziak. *Footwear Impression Evidence: Detection, Recovery and Examination*. 2<sup>nd</sup> ed. Boca Raton: CRC Press, 2000.
2. Herbert Bay, Tinne Tuytelaars, and Luc Van Gool. SURF: Speeded-Up Robust Features. *ECCV'06 Proceedings of the 9<sup>th</sup> European Conference on Computer Vision*, Graz, Austria, Part I: 404-417. 2006.
3. Nicole Richetelli, Madonna Nobel, William J. Bodziak, and Jacqueline A. Speir. Quantitative Assessment of Similarities Between Randomly Acquired Characteristics on High Quality Exemplars and Crime Scene Impressions via Analysis of Feature Size and Shape. *Forensic Science International*, 270: 211-222. 2017.
4. Soyoung Park. *Learning Algorithms for Forensic Science Applications*. PhD Diss., Iowa State University, 2018.

### Footwear Impressions, Similarity Score, Source





### B174 Estimating the Probability of Randomly Acquired Characteristics (RACs) Locations on a Shoe Sole

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**Learning Overview:** After attending this presentation, attendees will better understand the process of comparing the shoes of a suspect with prints left at a crime scene and the importance of faults on the shoe sole, known as RACs—especially their spatial distribution—in determining the findings' evidential value. Novel methods for estimating the probability of a RAC appearing at a given location on a shoe sole will be explained, as well as the impact of this information on the likelihood of a match.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by developing a greater expectation that forensic evidence have a solid scientific basis.

In recent years, forensic methods have been criticized for their shortcomings in providing courts with objective and quantitative answers to the question of whether a sample from a suspect matches a sample found at the crime scene. Unlike DNA that is used routinely to link suspects to crime scenes because of its scientific objectivity and accessible documentation, the evaluation of other types of evidence, such as shoeprints, tire tracks, tool marks, and the like, has not reached this gold standard. Both the 2009 National Research Council (NRC) Report, *Strengthening Forensic Science in the United States: A Path Forward*,<sup>1</sup> and the 2016 President's Council of Advisors on Science and Technology (PCAST) Report to President Obama, *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*, have called for the strengthening of the scientific basis of forensic procedures.<sup>1,2</sup>

Here, shoeprint comparison is considered. The identification of footwear impressions is based on the comparison of a print found at the crime scene with a print made from a suspect's shoe. The analysis of shoeprints by experts is done in two broad stages. First, the pattern, size, and wear of the shoe sole are compared to the crime scene print. If these do not fit, the analysis is stopped, and the pair is classified as a non-match. In the second stage, the forensic expert examines whether RACs on the shoe sole, such as scratches or holes, match the RACs on the print from the crime scene. These RACs may have various characteristics; for example, location, shape, and orientation. However, the distribution of RAC characteristics has not been investigated thoroughly. New statistical methods are presented for estimating the probability of a RAC at a given location on the shoe sole. RACs are assumed to follow a 2D point process, the intensity function of which governs the probability of their appearance at different locations. A smooth intensity function based on pixel data is first estimated using a logistic regression and natural cubic splines. This approach fully exploits the data, but may be problematic as the definition of location is ambiguous. An alternative approach is presented, which uses a piece-wise constant intensity function in regions defined by forensic experts. It is shown that RACs are likely to appear at certain locations, corresponding to the foot's morphology.

The findings of this study take us a step forward in assessing the evidential value of shoeprint comparison. As RACs in certain locations are rarer than in others, they carry more weight in determining the rarity of the shoe sole, or in other words, the probability of a match between the crime scene print and the suspect's shoe.

#### Reference(s):

1. National Research Council (NRC), Committee on Identifying the Needs of the Forensic Science Community. (2009). *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press.
2. President's Council of Advisors on Science and Technology (PCAST). *Report to the President: Forensic Science in Criminal Courts: Ensuring Scientific Validity Of Feature-Comparison Methods*. (2016). Washington, DC: Executive Office of the President of the United States.

#### Footwear Impression, Randomly Acquired Characteristics, Shoeprints



### B175 The Effects of Elevated Temperatures and Substrates on the Weathering of Ignitable Liquids

*Caitlyn Wensel, BS\*, West Virginia University, Morgantown, WV 26505; Isaac Willis, BS, Michigan State University, East Lansing, MI 48823-8325; Zilin Fan, PhD, Tianjin Fire Research Institute of MPS, Morgantown, WV 26506; J. Tyler Davidson, MS, West Virginia University, Morgantown, WV 26505; Glen P. Jackson, PhD, West Virginia University, Morgantown, WV 26506-6121*

**Learning Overview:** After attending this presentation, attendees will better understand how elevated temperatures, the type of substrate, and the penetration of the ignitable liquid into each substrate affect the relative evaporation rates of different components of the ignitable liquid. Attendees will better understand the different phenomena that lead to the observed weathering of ignitable liquids in fire debris.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of how the volatiles within a mixture evaporate at different rates when they are spiked onto different common household substrates and evaporated at elevated temperatures that mimic casework. Such understanding will assist analysts in making more reliable inferences on the presence and identification of ignitable liquids in authentic casework samples.

**Hypothesis:** Two central hypotheses are that the temperature at which weathering occurs and the penetration depth of an ignitable liquid into a porous substrate will both affect the relative evaporation rates of the components in the mixture. An additional hypothesis is that the surface chemistry of a non-porous substrate will not influence the weathering of an ignitable liquid.

**Methods/Results:** Analyses involved the development of a synthetic gasoline sample and a variety of sample treatments and weathering conditions. A simplified, synthetic form of gasoline was created using nine compounds that represent a wide range of volatilities and chemical classes in fresh gasoline, including alkanes, substituted aromatics, an indane, and a polyaromatic. To achieve the desired weathering, an aluminum block with an aluminum weight boat in the center was preheated to the desired temperature in a vacuum oven, while a second aluminum block was cooled to -20°C in a freezer. Once the heating block and weight boat reached the appropriate temperature, a measured aliquot of the synthetic gasoline was spiked into the weight boat and weathered at the desired temperature to the desired extent, at which time the weight boat was transferred to the cold block to prevent additional evaporation.

The five substrates included a hard wood, a soft wood, nylon carpet, olefin carpet, and cotton. To simulate the expected conditions of casework samples, different delay times were studied between when the ignitable liquid was spiked on each substrate and when the weathering was initiated. Penetration times ranged from 30 seconds to 30 minutes.

Preliminary results corroborate this evaporation model, which is based on equilibrium theory, in which the temperature of evaporation plays a major role in the relative distribution of the residues in weathered ignitable liquids. For example, when a synthetic gasoline sample is weathered 90% at 30°C, the molar fraction of toluene—the most volatile component in the mixture—decreases from the original value of ~35% to a value that is below the threshold for detection on the Gas Chromatography/Mass Spectrometry (GC/MS). However, when the same mixture is weathered to the same extent at 210°C, toluene is readily observed at a molar ratio of ~1% after weathering. The model shows that compounds of disparate boiling points have a narrower range of vapor pressures at elevated temperatures, which explains why the disparate compounds evaporate at more similar rates at elevated temperatures than at room temperature.

Experiments regarding the effect of substrate are still ongoing, but preliminary results show that short (e.g., 30 seconds) penetration times on different surfaces do not significantly influence the weathering pattern of the gasoline simulant. The effects of longer penetration times are currently undetermined.

#### **Ignitable Liquids, Weathering, Household Substrates**



### **B176 The Utilization of Portable Gas Chromatographic (GC) Systems Coupled With Capillary Microextraction of Volatiles (CMV) for On-Site Detection of Ignitable Liquid Residues (ILRs)**

Michelle N. Torres, BS\*, Florida International University, Miami, FL 33199; Nicole Valdes, BA, Florida International University, Miami, FL 33199; Jose R. Almirall, PhD, Florida International University, Miami, FL 33199

**Learning Overview:** After attending this presentation, attendees will be familiar with the performance capabilities of current generation portable Gas Chromatograph/Mass Spectrometers (GC/MS) as applied to fire investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the advantages and disadvantages of the systems and the extraction techniques presented here, which would be particularly helpful to fire scene investigators and fire debris analysts.

Laboratory methodologies for the detection and analysis of ILRs in fire debris are well-established; however, methodologies pertaining to on-scene detection and identification are less defined. Instrumentation, such as hydrocarbon “noses,” have not been widely utilized due to a lack of specificity and have instead been surpassed by ILR-trained canines. Thanks to recent advancements in miniaturized hyphenated systems, the potential for rapid and more accurate on-site detection of ILRs exists. With the growing availability of these portable systems on the market, there is now a need to evaluate their effectiveness in the field relative to traditional laboratory methods.<sup>1</sup>

In this study, two portable GC/MS systems, the TRIDION™-9 and the Griffin™ G510, are applied to the analysis of ignitable liquid residues recovered from fire debris. Both systems are low thermal mass GCs; the TRIDION™-9 utilizes a toroidal ion trap as its detector while the Griffin™ utilizes a linear quadrupole. The instrument inlets are configured to allow Solid Phase Microextraction (SPME) sampling in addition to other sampling techniques. Other commercially available accessories available from both instrument companies also allow for the coupling of the CMV device. The CMV is a dynamic headspace sampling device which consists of a dual open-ended capillary tube filled with glass microfiber strips that are coated in a Polydimethylsiloxane (PDMS) -incorporated sol-gel polymer. The CMV can be thermally desorbed by direct insertion into a GC/MS inlet and has had successful applications to several materials of forensic interest.<sup>2-4</sup>

The two instruments are evaluated using several points of comparison. Chromatographic resolution, software identification capabilities, overall time of analysis, and ease of use are evaluated. The figures of merit for each system are also compared to those of a benchtop GC/MS system. Method development and optimization is carried out using standardized accelerant mixtures. Simulated debris sampling is carried out in three scenarios: closed-system using 1L steel paint cans or paper cups with various charred substrates, and open-system using a calibrated vapor source. The techniques used for sampling include SPME fibers and the CMV with their recovery capabilities compared across all three systems. Finally, both systems have been utilized onsite at an accelerated live-burn exercise, and the results of this exercise are also presented.

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1. Almirall, J.; Arkes, H.; Lentini, J.; Mowrer, F.; Pawliszyn, J. Forensic Science Assessments: A Quality and Gap Analysis. *Fire Investigation*. 2017.
2. Tarifa, A., Kreitals, N.M., Mulloor, J., Gura, S., and Almirall, J.R. 2017. Cryofocusing Capillary Microextraction of Volatiles (Cryo-CMV) as a Novel Headspace Extraction Device for the Analysis of Volatile Organic Compounds and Smokeless Powders. *Forensic Chemistry*. 3:81–89.
3. Wiebelhaus, N., Hamblin, D., Kreitals, N.M., and Almirall, J.R. Differentiation of Marijuana Headspace Volatiles from Other Plants and Hemp Products Using Capillary Microextraction of Volatiles (CMV) Coupled to Gas-Chromatography–mass Spectrometry (GC–MS). *Forensic Chemistry*. 2016. 2: 1-8.
4. Gura, S., Tarifa, A., Mulloor, J., Torres, M.N., and Almirall, J.R. Capillary Microextraction of Volatiles Device for Enhanced BTEX Vapors Sampling Based on a Phenyl Modified PDMS Sol-Gel Adsorption Phase. *Analytica Chimica Acta* 2018.1014:27–40.

#### **Fire Debris Analysis, Headspace Sampling, Portable GC/MS**



### B177 The Benefits of High-Resolution Mass Spectrometry for the Identification of Ignitable Liquids

Haylea Debolt\*, State College, PA 16801; Frank Dorman, PhD, University Park, PA 16802-4500

**Learning Overview:** After attending this presentation, attendees will see that through the use of high-resolution mass spectrometry, ignitable liquids may be more readily identified as well as the estimation of the degree of weathering (or loss) as compared to traditional methodologies employing nominal-mass mass spectrometry.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing forensic scientists with a more precise method of identifying specific ignitable liquids.

The current method used to identify ignitable liquids consists primarily of pattern matching either the gas chromatogram or extracted-mass chromatograms of the unknown sample to the gas chromatogram or extracted-mass chromatograms of a reference sample. This is performed by computer software in conjunction with the analyst and is subject to possible bias, which may impact the results. The samples are also likely to undergo significant degradation and experience interferences due to evaporation, microorganism activity, matrix effects, or from the actual combustion. The potential degradation and interferences can remove or add compounds, which may further impact the identification ability. These missing or additional compounds can negatively impact the ability to properly match the obtained pattern to that of the reference sample.

Additionally, when comparing the mass spectra between the unknown and reference samples using common analytical approaches, only nominal mass (integer mass) is observed. Fractional mass differences can be utilized to correctly distinguish between the fragments in a sample, which in turn can be used to better identify the compounds in the questioned ignitable liquid. These identified compounds can then be used to classify the ignitable liquids by American Society for Testing and Materials (ASTM) standard practices. Having a method that can identify specific compounds gives more validity to identifying ignitable liquid samples than the standard pattern-matching practices. A quantitative element is gained, and the possibility of bias and misidentification by the analyst is reduced. The goal of this project is to demonstrate that high-resolution mass spectrometry can be used to better identify ignitable liquids based upon fragments fractional-mass values.

Known ignitable liquid samples were analyzed using a LECO® Pegasus® GC-HRT+ 4D High Resolution Time-Of-Flight/Mass Spectrometry (TOF/MS). Reference samples were used to construct an accurate-mass library. The data collected underwent statistical analysis in order to group together relevant fragments that could be useful in identification. Then, the statistical data along with the library were used to identify unknown ignitable liquid samples. These unknown samples included both neat and weathered samples. Preliminary results showed that this technique would prove useful in the identification of ignitable liquids.

#### **Ignitable Liquids, High-Resolution Mass Spectrometry, Gas Chromatography**



### **B178 A Foundational Study of Fire Debris Interpretation Using Quantitative Measures of Chromatographic Features**

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**Learning Overview:** The goal of this presentation is to introduce a validated method that quantifies the data supportive of a gasoline identification using a sufficiency chart and to introduce documentation software that allows for a more transparent inferential process and is capable of verification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a quantitative sufficiency chart and a computerized pattern-comparison documentation program that also facilitates verification that will make the fire debris experts' inferential process standardized, objective, and visible.

This presentation will discuss an interpretation method for fire debris Gas Chromatography/Mass Spectrometry (GC/MS) data using quantitative measures based on key chromatographic features, which were used to establish the sufficiency of the data for a conclusion. The analysis of the key chromatographic features is highlighted, as well as the process by which a graphical display of the sufficiency of the data was created. This novel approach to data interpretation is the first step toward a validated analysis method for ignitable liquids, starting with gasoline, in the presence of complex matrices. Ultimately, this research aims to make the fire debris analysis process more standard, objective, and transparent by establishing a validated method with quantitative measures that includes the implementation of intuitive documentation software and verification, all of which is based on robust data evaluation.

Ignitable liquid identification is based on pattern recognition techniques using chromatographic data. While the analytical processes associated with the analysis of fire debris for the presence of ignitable liquids are based on fundamental chemical properties, the interpretation process is entirely subjective. The degree of subjectivity is directly related to the abundance and type of interferences from volatile compounds produced by the matrix (including inherent, pyrolysis, and combustion products), as well as the concentration of any ignitable liquid residues present in the sample. Currently, there are no published, validated methods for the interpretation of these complex samples, which can, and does, lead to a lack of consistency in interpretation and reporting within the field. Therefore, this study was designed to establish a novel quantitative measure of assessing the chromatographic features of gasoline generated during analysis by applying statistical measures to create sufficiency parameters for use in interpreting data and rendering conclusions.

A methodology for establishing and measuring variables associated with quantity, quality, and confidence is being optimized and applied to neat gasoline samples, gasoline samples evaporated at 25%, 50%, 75%, and 90% levels, negative matrix samples, and real-world samples composed of mixtures of matrix and gasoline. Key chromatographic features in 150 gasoline samples were evaluated and 64 chromatographic peak height ratios determined for comparison. Statistical analysis was conducted to determine the variation observed for each of these ratios in the known gasoline samples and to determine the presence of these key chromatographic features in the negative matrix samples. This information was evaluated to determine the relative scores for each of these features. The scores were used to create a sufficiency chart, which is a graphical display detailing the totality of data supporting a potential gasoline identification. The sufficiency chart will also identify the "gray area" where analysts are more likely to form differing opinions. A discussion of this "gray area," its implications, and the proposed solutions under development will be presented.

The final results of this study will be a foundational validation with essential quality assurance measures for the purpose of identifying gasoline in fire debris samples. The investigators plan to use methodologies similar to Analysis/Comparison/Evaluation/Verification (ACE-V) for documenting the examination process, thereby ensuring greater transparency in fire debris examination and comparisons. However, this documentation process that will be introduced is currently under development. The end-goal of the proposed project is to generate a quantitative sufficiency chart for reliable data interpretation with the adaptation and utilization of a computerized pattern-comparison documentation program that also facilitates verification.

#### **Fire Debris Analysis, Gasoline, Interpretation Method**



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### **B179 Human Scent Biometrics: Paving a Path Toward Improved Data Analysis**

*Vidia A. Gokool, BS\*, Miami, FL 33157; Howard K. Holness, PhD, Miami, FL 33199; Kenneth G. Furton, PhD, Florida International University, Miami, FL 33199*

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**Learning Overview:** After attending this presentation, attendees will understand the hurdles facing the use of human scent as a biometric and the Gas Chromatography/Mass Spectrometry (GC/MS) data processing approaches being implemented toward the systemization of scent profile comparisons.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by equipping attendees with approaches for extrapolating the influential features of their GC/MS data, assessing the appropriateness of statistical tests, and venturing into exploratory data analysis as presented through the lens of human scent profiling.

Human scent is a complex mixture of Volatile Organic Compounds (VOCs) detected in the headspace above a scent sample. These VOCs are the culmination of oils, sweat, and other skin secretions enduring microbial action at the skin's surface. With genetic and environmental influences each playing a role in its characteristics, human odor is a product of diverse factors that allow it to be viewed as a biometric that can be used to identify a person.

There is a seeming agreement arising in regard to best practices for human scent collection with multiple reported uses of Headspace/Solid Phase Microextraction (HS/SPME) and wide utilization of Gas Chromatography/Mass Spectrometry (GC/MS) to identify the VOCs extracted from human scent samples. Despite convergences in approaches toward the collection and instrumental analysis, there is no commonly accepted practice for interpreting the resulting GC/MS profile data.

Utilizing hand odor profiles retrieved by HS/SPME-GC/MS efforts to develop a robust systematic approach for human scent profile discrimination will be examined. With regard to previously implemented statistical tests, such as Spearman Rank Correlation, Linear Discriminant Analysis (LDA), and Principal Component Analysis (PCA), additional considerations also have to be evaluated for their utility in defining class barriers and making determinations of association between profiles. Inherent to the improvement of existing approaches, an expanded evaluation of chromatographic feature relevancy has been conducted. Namely, elution time, peak height, and peak area were compared in relation to mass spectral compound identifications to assess the influence of each parameter on profile comparison. The resulting methods for performing human hand odor profile discrimination are presented in conjunction with paths for reducing data complexity and conducting an exploratory investigation of the processed data; with topics including multivariate analysis and clustering methods.

Data analysis continues to be a roadblock between the academic endeavor of human scent profiling and the casework application of this technique. Through further developing the presented methods, a path will continue to be paved between this novel method and the crime laboratory where it may be utilized.

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#### **SPME-GC/MS, Human Scent, Data Analysis**



### **B180 The Practical Application of a Kinetic Model to Generate Predicted Reference Collection for the Identification of Ignitable Liquids in Fire Debris Samples**

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**Learning Overview:** The goal of this presentation is to demonstrate the practical application of a kinetic model to generate a reference collection of predicted chromatograms used to identify ignitable liquids in fire debris samples.

**Impact on the Forensic Science Community:** After attending this presentation, attendees will understand how the kinetic model can be applied to the chromatogram of an unevaporated liquid to predict chromatograms corresponding to a range of evaporation levels. These predicted chromatograms form the basis of an extensive reference collection which, in this work, is used to identify ignitable liquids in samples collected from large-scale burns.

Fire debris samples are analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) to determine the presence of ignitable liquids based on comparisons to a suitable reference collection. Identifications can be challenging due to evaporation of the ignitable liquid and the presence of interferences arising from substrates in the fire debris samples. These challenges can be overcome by including evaporated liquids in the reference collection and by using extracted ion profiles to minimize or eliminate substrate effects. However, experimental evaporations can be time consuming, with the result that not all liquids in the reference collection will be evaporated to different levels.

A first-order kinetic model was previously developed in the Michigan State University laboratory to predict evaporation rate constants for compounds in ignitable liquids as a function of retention index, which is related to boiling point. The evaporation rate constants are used to calculate the fraction remaining of each compound in a liquid, from which chromatograms corresponding to evaporated liquids can be predicted. Previous work in this laboratory demonstrated the predictive accuracy of the model for petroleum distillates, whereas recent work highlighted improvements in accuracy for gasoline. In this work, practical application of the model is demonstrated.

The kinetic model was applied to generate a reference collection of ignitable liquids from five different chemical classes defined in American Society for Testing and Materials (ASTM) 1618 (isoparaffinic, aromatic, naphthenic-paraffinic, petroleum distillate, and gasoline). Unevaporated liquids from each class were analyzed by GC/MS to generate Total Ion Chromatograms (TICs) and Extracted Ion Profiles (EIPs) of the major compound classes. The kinetic model was applied to the TICs to predict chromatograms corresponding to total fraction remaining ( $F_{Total}$ ) levels of 0.9–0.1 (evaporation levels of 10%–90%) for each liquid. Predicted reference collections corresponding to the EIP for major compound classes were generated in a similar manner.

The liquids were also experimentally evaporated to different  $F_{Total}$  levels and analyzed by GC/MS. The TICs and EIPs of the experimentally evaporated liquids were then compared to the corresponding predicted reference collection, using Pearson Product-Moment Correlation (PPMC) coefficients as a measure of similarity. For example, for the TIC of paint thinner experimentally evaporated to  $F_{Total}=0.50$ , strongest correlation was observed for comparisons to predicted TICs of paint thinner, with a maximum PPMC of 0.942. Conversely, the PPMC coefficients for comparisons of the experimentally evaporated paint thinner to other liquids in the predicted reference collection were generally less than 0.500, indicating weak correlation.

To demonstrate practical application of the model, three burn cells (8ft x 16ft) furnished with carpeting and other household items were prepared. Gasoline was poured in two of the cells and paint thinner in the third cell. Each cell was ignited and allowed to burn for 8min or just past flashover. The fires were extinguished, and samples were collected and analyzed by GC/MS. The resulting TICs and EIPs were compared to the predicted reference collections.

For burn samples containing paint thinner, the maximum PPMC coefficient for comparison to the TIC reference collection was 0.862, which corresponded to the predicted TIC of paint thinner with  $F_{Total}=0.80$ . Higher correlation was observed when the EIPs were considered. For example, the maximum PPMC coefficient of 0.935 was observed for comparison to the cycloalkanes/alkenes EIP with  $F_{Total}=0.90$ . Only weak correlation was observed for comparisons to all other liquids in the TIC and EIP reference collections, with PPMC coefficients less than 0.500.

This presentation will describe the development of the predicted reference collection in more detail and application to data collected from large-scale burns. Overall, this work will demonstrate the utility of the kinetic model combined with EIPs to more accurately predict the presence of ignitable liquids in fire debris samples.

#### **Fire Debris, GC/MS, Extracted Ion Profile**



### **B181 Expert System for Characterization Using AMDIS Plus Excel® (Escape): Connecting Cultural Heritage Research to Trace Evidence Analysis by Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS)**

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**Learning Overview:** After attending this presentation, attendees will be aware of the technology and expert database used by cultural heritage scientists.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing details about the automated system for evaluating complex Py-GC/MS data, resulting in a more detailed classification of paint-binding media.

Cultural heritage research shares several things in common with the field of trace evidence analysis. Both are interested in learning as much as possible about the major, minor, and trace components present in tiny samples originating from a diverse range of materials. Sample characterization typically involves Fourier-transform infrared spectrometry, Raman spectroscopy, scanning electron microscopy with energy-dispersive X-ray spectrometry, and polarized light microscopy. For in-depth compositional information about paints, coatings, adhesives, plastics, and a range of other organic materials used by artists—and to learn how the materials have changed over time—cultural heritage scientists routinely turn to Py-GC/MS. Microfurnace pyrolyzers possess many advantages over other pyrolyzer types, including an ambient temperature sample introduction area purged with helium, a deactivated quartz furnace tube, a reproducible pyrolysis event ensured by sample cups dropping into a pre-heated zone, and mounting the furnace directly onto the GC inlet to minimize problems of cold spots.

New practitioners of Py-GC/MS face a number of daunting challenges: locating relevant information scattered throughout disparate literature, selecting an optimum analytical method, recognizing material types that require derivatization, processing hundreds of GC peaks, accurate compound identification when mass spectral libraries are incomplete, recognizing characteristic patterns of marker compounds that relate to specific materials, proper characterizing material mixtures, and the need for data presentation that is meaningful to non-scientists. As interpretation of Py-GC/MS data requires considerable expertise, many potential users in both disciplines avoid learning the technique.

The Expert System for Characterization using Automated Mass spectral Deconvolution and Identification System (AMDIS) Plus Excel® (ESCAPE) was developed at the Getty Conservation Institute (GCI) as a two-step Py-GC/MS data processing tool to identify compositions of decorative lacquers, coatings, and paints. AMDIS (from the National Institute of Standards and Technology [NIST]) is used with a specialized 1,500 compound library to identify marker compounds in sample data. Specialized Excel® reports sort the AMDIS marker compound report by material type and provide users with expert knowledge needed for confirming the presence of materials in the sample. Expert knowledge in ESCAPE originates from interviews of Py-GC/MS experts in material characterization and from publications. Excel® reports present the analytical results in various ways—marker compound tables, diagnostic bar graphs, pie charts of composition, and correlation coefficient tables of marker compounds—to best convey the results to both practitioners and non-scientists. Shared databases of the final analytical results provide benefit to the entire cultural heritage community.

In order to evaluate the accuracy of ESCAPE and its applicability to forensic paint samples, Collaborative Testing Services (CTS) samples were tested at the GCI in a collaboration with trace evidence examiners from the Los Angeles County Sheriff's Department. The results were fully consistent with the known compositions, and compositional differences between Q and K specimens were clearly illustrated by bar graphs of key markers in the binding media. Additional studies of the diverse types of paints used in the creation of art—tube colors, house paints, industrial paints, and spray paints—illustrated best practices for clear presentation of compositional data for acrylic, vinyl, alkyd, epoxy, and urethane binding media.

ESCAPE could be considered as a common platform for sharing paint composition data between the forensic and cultural heritage research communities. It can be customized to meet the unique needs of the forensic community, especially in regard to developing meaningful formats for presenting analytical results in court. Interested researchers learn ESCAPE in workshops conducted by the GCI, so that researchers with Py-GC/MS instrumentation can immediately start applying ESCAPE in their work. For laboratories without a Py-GC/MS, ESCAPE provides justification for equipment purchase as the expert knowledge in the system overcomes the steep learning curve for new users. Collaboration with experts in toxicology, arson, tapes, and fibers can extend the utility of ESCAPE beyond paints and coatings. One desired outcome is the development of a shared paint composition database that would benefit the cultural heritage and forensic communities on both a national and an international level.

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#### **Py-GC/MS, Paint Analysis, Shared Database**





### **B182 A Comparison of Direct Analysis in Real-Time High Resolution Mass Spectrometry (DART®-HRMS) and Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) in Automotive Paint Analysis**

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**Learning Overview:** After attending this presentation, attendees will understand the benefits of using DART®-HRMS in comparison to Py-GC/MS when analyzing automotive paint evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a rapid ionization technique for the analysis of automotive paint evidence. Furthermore, a comparison of the discriminatory capabilities of both techniques will be demonstrated.

Currently, there are three common analytical techniques used for the analysis of automotive paint: microscopy, infrared spectroscopy, and Py-GC/MS. Py-GC/MS is the gold standard when it comes to automotive paint analysis due to its ability to differentiate between paint samples that are indistinguishable by infrared spectroscopy. The reason for the high discriminatory capability of Py-GC/MS is the technique's sensitivity to binders, additives, and cross-linking agents. However, a disadvantage of Py-GC/MS is that the technique is time consuming and destructive to actual case samples.

A preliminary study determined that DART®-HRMS could discriminate black car paint samples and provided comparable results to Py-GC/MS.<sup>1</sup> Therefore, a larger study was conducted to determine if DART®-HRMS provides better or comparable discriminatory results to Py-GC/MS while also having the advantage of analyzing samples in a fraction of the time. DART®-HRMS is a rapid screening technique that uses soft ionization. The technique requires little sample preparation and rapidly analyzes samples in four minutes under ambient conditions. Moreover, DART®-HRMS has the capability to measure the mass of large and high-weight molecules, such as polymers commonly found in automotive paints. Both techniques were compared to assess how the data obtained from DART®-HRMS related to that of Py-GC/MS.

A cross-section of the paint was cut with a scalpel from 100 samples that were obtained from automotive body shops around the Orlando, FL, area and from a junkyard in Pembroke Pines, FL. A VHX 6000 Keyence digital microscope was utilized to visualize the cross sections and determine layer systems of each sample. The clear coat and base coat of the 100 samples were analyzed using DART®-HRMS in positive ionization mode. Several samples were analyzed, in triplicate, in negative ionization mode on the DART®-HRMS; however, no additional information was obtained. The clear coats of the set of 100 samples were also analyzed in duplicate with Py-GC/MS for comparative purposes. Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and Linear Discriminant Analysis (LDA), were performed on the data to determine the classification potential of each instrumental technique. HCA was performed to identify unsupervised clusters within the data. Then, PCA was utilized to reduce the dimensionality of the data set to make it easier to visualize the patterns in each dataset. Last, LDA was performed to determine the accuracy of the models. The multivariate statistics performed on the data demonstrated that DART®-HRMS can be as discriminating as Py-GC/MS. DART®-HRMS has the potential to be a great addition to the analytical techniques currently used for the analysis of automotive paint due to the method's rapid analysis time.

*This research was supported by the 2017 Forensic Science Foundation's Lucas Grant.*

#### **Reference(s):**

<sup>1</sup> Maric, M.; Marano, J.; Cody, R.B.; Bridge, C. DART-MS: A New Analytical Technique for Forensic Paint Analysis. *Analytical Chemistry* 2018.

**Automotive Paint, DART®-HRMS, Py-GC/MS**



### **B183 Barking Up the Wrong Tree: Detection and Identification of Illegally Traded Endangered Species of Wood Using Mass Spectral Techniques**

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**Learning Overview:** The goals of this presentation are to introduce attendees to: (1) field-optimal sample collection for detecting and identifying illegally traded endangered species of wood; (2) classification of wood species using multivariate statistical analysis processing of mass spectral data; and (3) identification of diagnostic molecules for the identification of illegally traded wood species.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on a technique that enables law enforcement to distinguish and recognize endangered wood species in open spaces, such as shipping containers or at crime scenes.

Illegal logging and related trade occur when timber is harvested, transported, processed, bought, or sold in violation of national or sub-national laws. Globally, the act of illegal logging nets billions of dollars annually and accounts for 70% of all timber exports for some countries.<sup>1</sup> Organized crime networks in conjunction with corrupt government officials contribute to these sobering statistics. The illegally traded logs are difficult to distinguish visually from legally traded wood, and conventional identification techniques, including DNA profiling, morphological feature characterization, and stable isotope analysis, are time-consuming, costly, and require specialized expertise. These challenges make it more difficult for law enforcement to detect and identify illegally traded woods at points of entry into the United States. Therefore, a technique that is more readily amenable to field sampling is needed that will allow for the rapid detection and identification of illegally traded endangered woods. This study demonstrates that species-level identification can be achieved through the use of species-specific chemical signatures revealed by Direct Analysis in Real Time High Resolution Mass Spectrometry (DART®-HRMS) along with Gas Chromatography/Mass Spectrometry (GC/MS). The results demonstrate proof-of-concept that if a database of species-specific chemical signatures of endangered woods could be created, samples collected in the field can be screened against it to enable rapid species identification.

To develop the approach, multiple different species of wood representing a range of genera were shredded into a fine powder. The headspace volatiles of each were concentrated onto conditioned Solid Phase Microextraction (SPME) fibers for 30 minutes. The fibers were then analyzed using DART®-HRMS by suspending the fiber directly in the DART® gas stream for 30 seconds. The powdered woods were also analyzed directly by placing the closed end of a melting point capillary tube into the material, then suspending the coated surface of the tube in the DART® gas stream. The DART® mass spectra consistently showed inter-species differences and intra-species similarities and, therefore, the data were subjected to multivariate statistical analysis processing in order to perform classification. A set of feature masses were selected as species-specific diagnostic  $m/z$  values for each set of results, and these were used to perform kernel discriminant analysis. The statistical analysis processing showed that classification of wood species could be achieved from the chemical fingerprints produced from the direct analysis of the bulk material as well as the headspace of wood samples. In order to identify the molecules responsible for the ability to discriminate between species, the samples were analyzed using Thermal Desorption-Gas Chromatography/Mass Spectrometry (TD-GC/MS). The results revealed the identities of several  $m/z$  values whose presence was diagnostic of species, including 107.0492, 133.0663, and 137.0619 for the headspace samples and 255.1016, 285.1100, and 375.1097 for the bulk material samples. Tentative identification could be made in some cases and revealed the presence of benzaldehyde, cinnamaldehyde, and 4-methoxybenzaldehyde in the headspace and dalbergichromene, 3,4-dimethoxydalbergione, and caviunin in the bulk material. These results indicate proof-of-concept that the mass spectral analysis of wood samples can be used to create a database available to border patrol agents that can be used for the efficient identification of illegally traded wood species.

#### **Reference(s):**

- <sup>1</sup> Nellemann, C.; Henriksen, R.; Raxter, P.; Ash, N.; Mrema, E. *The Environmental Crime Crisis—Threats to Sustainable Development from Illegal Exploitation and Trade in Wildlife and Forest Resources*. United Nations Environmental Programme and GRID-Arendal, Nairobi and Arendal: 2014.

#### **Endangered Wood, Species Identification, Mass Spectrometry**



### **B184 The Contamination of Homes With Methamphetamine: Are Current Assessment and Remediation Approaches Adequate?**

Stewart Walker, PhD\*, Flinders University, Adelaide, South Australia 5001, AUSTRALIA; Kirstin Ross, PhD, Flinders University, Adelaide 5042, AUSTRALIA; Jackie Wright, PhD, Environmental Risk Sciences Pty Ltd, Sydney, NSW 2118, AUSTRALIA

**Learning Overview:** The goal of this presentation is to inform attendees on the effectiveness of the current assessment of risk from living in homes contaminated with methamphetamine and the effectiveness of different remediation processes over time.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving understanding of the effectiveness of different remediation processes for removing, or covering up, methamphetamine contamination in a variety of materials found in domestic homes, including walls, ceilings, soft fabrics, blinds, and toys.

Contamination of domestic dwellings from methamphetamine cooking or smoking is an increasing public health problem in many countries.<sup>1,2</sup> To evaluate the extent of contamination, sampling generally focuses on collection of surface-wipe samples from walls and other surfaces of a potentially contaminated home and analysis by established methods. However, the concentration of methamphetamine on surfaces may not give a true representation of the extent of contamination. The contamination levels of many household materials may depend on the material, the porosity, absorptivity, and the room in which the material was located with respect to the room where the drugs were synthesized or smoked. Results in this presentation come from a long-term study of contamination in a home (Rural Victoria, Australia) that had been used to cook methamphetamine, then sold, lived in for 18 months by the new owners until it was deemed to be unfit for human habitation, then left unattended (since March 2015).<sup>3</sup> Although the time since the cooking had taken place was significant (more than six years), the levels of contamination were still extremely high in items that were part of the house when the cooking of drugs was taking place (blinds, carpets, walls). In addition, levels were also high in articles brought to the house post-cooking (rugs, toys, beds, etc.). Both wipe sampling and analysis of bulk samples indicate that the methamphetamine is not breaking down or being removed and is being transferred from contaminated to non-contaminated objects.

Furthermore, the concentration of methamphetamine inside the boards (plasterboard—a building material made from calcium sulfate dehydrate sandwiched between paper) of the ceiling were found, in places, to be around three times higher than the concentration on the external surface, indicating that the methamphetamine had soaked in.

A study of several remediation processes was also carried out in this property with “fogging,” “ozone,” “washing,” and painting over with oil-based and water-based paints applied to large sections of the wall. Initial results indicated a reduction in the level of methamphetamine detected; however, in all cases, methamphetamine was still detected over time in subsequent analysis. If the level of methamphetamine inside the wall is not reduced by these surface remediation treatments, then it may seep out over time, leading to “recontamination” and exposure to people living in the “treated” house. The same applies to methamphetamine absorbed into materials, rugs, toys, beds, etc.

These results raise questions about the adequacy of characterizing contamination and making decisions about the extent of remediation required, based just on surface-wipe samples. Without fully understanding the extent of contamination that is present, not only on surfaces, but within the building materials, it is difficult to ensure that the correct and most effective remedial approaches are taken to appropriately address the risks to inhabitants.

#### **Reference(s):**

1. Wright, J.L., Edwards, J.W., and Walker G.S. Exposures associated with clandestine methamphetamine drug laboratories in Australia, *Reviews on Environmental Health*, 2016, 1 31 (3) 329-352.
2. Gluckman, P. 2018. *Methamphetamine contamination in residential properties: Exposures, risk levels, and interpretation of standards*. New Zealand: Office of the Prime Minister's Chief Science Advisor. (Available from <https://www.pmcsa.org.nz/wp-content/uploads/Methamphetamine-contamination-in-residential-properties.pdf>).
3. Wright, J.L., Kenneally, M., Edwards, J.W., and Walker G.S. Adverse Health Effects Associated with Living in a Former Methamphetamine Laboratory—Victoria, Australia, 2015. *MMWR—Morbidity and Mortality Weekly Report*, 2017, Vol 65 Issue 52 1470-1473.

#### **Methamphetamine, Contamination, Remediation**



### **B185 Multivariate Statistics for the Masses: A Novel, User-Friendly Method for Positional Isomer Differentiation Using Mass Spectral Data**

*Jennifer Bonetti, MS\*, Virginia Department of Forensic Science, Norfolk, VA 23510*

**Learning Overview:** After attending this presentation, attendees will be aware of a novel method of positional isomer differentiation using only mass spectral data that is commonly generated during the analytical scheme of controlled substances. Additionally, attendees will be introduced to an easy-to-use user interface that has been developed with the typical forensic scientist in mind.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing an alternative method of positional isomer differentiation using multivariate statistical analysis of mass spectral data rather than other, more time-consuming methods, such as additional chemical or instrumental analysis.

Mass spectral analysis is the confirmatory method of choice for seized drug analysis in forensic laboratories. However, spectra of positional isomers are often too visually similar to allow for differentiation, which leads to identification challenges for forensic laboratories that are inundated with novel psychoactive substances. Scientists now find themselves in a position to make a choice between reporting an ambiguous conclusion that does not identify the specific isomer or resorting to additional chemical or instrumental analysis.

This presentation builds on previously published work that used multivariate statistical analysis of Fluoromethcathinone (FMC) and fluorofentanyl to offer a feasible alternative means of differentiating between electron ionization mass spectra of positional isomers, saving laboratory resources while still resulting in specific compound identification.<sup>1</sup>

Mass spectral data was collected from the four state regional laboratories across the Commonwealth of Virginia. Data from the past two to three years for any primary standards of commonly encountered positional isomers was requested. Isomer groups included pseudoephedrine/ephedrine, novel cathinone isomers, and several fentalogs. All data was collected on instruments that were tuned daily using either an autotune or etune procedure. A starting library of more than 950 spectra was collected from more than ten different Gas Chromatography/Mass Spectrometry (GC/MS) instruments.

The isomer groups were analyzed using the proposed method of spectral comparison which utilizes Principal Component Analysis (PCA) for dimension reduction, followed by Linear Discriminant Analysis (LDA) for isomer classification. For PCA, the original variables were the individual  $m/z$  ratios, and the data for each sample was its normalized abundance at each particular  $m/z$  ratio. The variables that were used to derive the LDA discriminant functions are the scores that resulted from projecting the original  $m/z$  variables onto the first several principal component axes. A user interface was developed that allows the user to dictate the number of principal components to utilize for LDA. Once selected, a Leave-One-Sample-Out Cross Validation (LOOCV) error rate can be calculated that shows how accurately the method can classify an unknown sample based on the available library.

Once the library is shown to be robust by resulting in a LOOCV error rate below a chosen threshold, the user interface allows a chemist to upload an unknown spectrum as a text file for comparison to the chosen group of isomers, and the program will use the above method to classify the compound based on the posterior probability. A blind study was conducted to show the accuracy of this classification method.

The results indicate multivariate statistical analysis, especially when combined with an easy-to-use user interface, is a promising addition to the analytical scheme of the identification of positional isomers. This would allow for higher confidence in the final identification of a compound without the need for additional chemical or instrumental analysis, saving laboratories both time and money.

#### **Reference(s):**

<sup>1</sup>. Bonetti J. Mass spectral differentiation of positional isomers using multivariate statistics. *Forensic Chemistry* 2018;9:50-61.

**Designer Drugs, Mass Spectrometry, Multivariate Statistics**



### B186 On-Scene Detection of Low-Dose Fentanyl Tablets

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**Learning Overview:** After attending this presentation, attendees will understand the capabilities and limitations of field detection and identification of fentanyl in low-dose tablets using commercially available portable instruments.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science and law enforcement community by establishing the best methods for field detection and identification of fentanyl in low-dosage forms.

Concerns about the illegal trafficking of fentanyl into the United States have largely been centered on its addition, in relatively pure powder form, to heroin and other illicit street drugs. These street drugs are dangerous to the end user, emergency responders, and the public at large. Due to the high potency of fentanyl (100 times that of morphine), a new trend is emerging in the illicit drug trade with the manufacture, smuggling, and distribution of relatively low concentrations of fentanyl dosage forms, including low-dose fentanyl tablets. The United States Drug Enforcement Administration (DEA) started a Fentanyl Signature Profiling Program (FSPP), analyzing samples from fentanyl seizures to help identify the international and domestic trafficking networks responsible for many of the drugs fueling the opioid crisis. In 2017, the FSPP analyzed 520 fentanyl powder samples from seizures totaling 960kg of fentanyl. While the average purity was 5%, the DEA has indicated that fentanyl shipped directly from China often has purity levels above 90%, while fentanyl trafficked over the Southwest border from Mexico often has purity levels below 10%. For low-dose fentanyl tablets, the concentration has been estimated to be closer to 1%. These low-dose fentanyl tablets can pose a significant analytical challenge for the on-scene detection and identification of the fentanyl by law enforcement due to interference by other components of the tablet with the fentanyl chemical signature. In some instances, particularly with Infrared (IR) and Raman analytical methods, the spectral features of fentanyl become hidden behind the spectral features of the tablet's other components. This effectively masks the fentanyl signature and prevents the identification of the fentanyl when spectra are compared against library databases. In other cases, as with ion mobility spectrometry and high-pressure mass spectrometry, the fentanyl signature may be suppressed by the presence of the other components of the tablet. Additionally, there have been instances where the low concentration of fentanyl-doped tablets has been assumed to be lower than portable instruments routinely deployed to field users are capable of detecting. To date, there has not been research that compared the detection capabilities for fentanyl when this substance is dispersed in typical pharmaceutical matrices across portable-instrument platforms.

This research analyzed fentanyl-doped acetaminophen samples using various commercially available portable instruments including IR spectrometers, Raman spectrometers, Surface-Enhanced Raman Spectrometers (SERS), mass spectrometers, gas chromatography/mass spectrometers, ion mobility spectrometers, and field-based color tests. Each technique's utility for the detection and identification of fentanyl was compared, with a focus on determining limits of detection when the fentanyl is dispersed at low concentrations within complex matrices. It was concluded that neither field-based color tests nor portable vibrational spectrometers have the limit of detection capabilities to positively identify fentanyl in these low-dose tablets. Analysis using SERS lowered detection limits. Additionally, ion mobility and mass spectrometers (with and without gas chromatography) were able to detect and identify fentanyl at concentrations less than those routinely found in the low-dose tablets (1%); however, each of these portable instrumental technologies have their own challenges that must be considered when completing this analysis.

#### Fentanyl, Field Detection, Low-Dose Tablets



### **B187 Mixed Illicit Drug Separation and Detection for the Point-of-Interdiction**

*Killian O'Connell, BS\*, Charlottesville, VA 22903; Michael Shane Woolf, MS, Charlottesville, VA 22903; James P. Landers, PhD, University of Virginia, Charlottesville, VA 22904*

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**WITHDRAWN**



## B188 On the Fragmentation Behavior of Fentanyl and Its Analogs in Electrospray Ionization-Tandem Mass Spectrometry (ESI-MS/MS)

J. Tyler Davidson, MS\*, West Virginia University, Morgantown, WV 26505; Zachary J. Sasiene, BS, West Virginia University, Morgantown, WV 26506; Younis F. Abiedalla, PhD, Auburn University, Auburn, AL 36830; Randall Clark, PhD, Auburn University, Auburn, AL 36849; Jack DeRuiter, PhD, Auburn University School of Pharmacy, Auburn, AL 36849; Glen P. Jackson, PhD, West Virginia University, Morgantown, WV 26506-6121

**Learning Overview:** After attending this presentation, attendees will better understand the structures and mechanisms of formation of at least three distinct isomeric/isobaric fentanyl product ions observed at nominal mass  $m/z$  188 in ESI-MS/MS. Attendees will learn about the concepts of multistage Mass Spectrometry ( $MS^n$ ), accurate mass measurements with High Resolution Mass Spectrometry (HRMS), and isotopic labeling for structural elucidation of fentanyl and Fentanyl-Related Compounds (FRCs).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of the fragmentation pathways of fentanyl and FRCs. Specifically, the identification of a novel isobaric fentanyl product ion at  $m/z$  188 with ESI-MS/MS will enlighten this community about potential issues with the use of the  $m/z$  188 fentanyl product ion for quantification with Multiple Reaction Monitoring (MRM).

**Hypothesis:** The central hypothesis is that the combination of  $MS^n$ , isotope labeling, and accurate mass measurements with HRMS will result in a deeper understanding of the fragmentation behavior of fentanyl and its analogs. Another hypothesis postulates that isomers having identical masses and different constitutional arrangements of atoms will provide measurable differences in their fragmentation patterns. A final hypothesis is that isotopic labeling (e.g., D for H and  $^{13}C$  for  $^{12}C$ ) does not alter the fragmentation behavior of an ion in a meaningful way, other than by changing the  $m/z$  value(s) of the product ions that contain the isotope(s).

**Methods/Results:** Analyses involve the characterization of fragmentation pathways of fentanyl and FRCs using  $MS^n$ , accurate mass measurements with HRMS, and isotopic labeling. Specifically, the identification of isobaric fentanyl product ions of the base peak at  $m/z$  188 with ESI-MS/MS was accomplished through the analysis of fentanyl, fentanyl-d5, and two  $^{15}N$ -fentanyl analogs. All compounds were analyzed using a Thermo Scientific™ LTQ™ Velos Pro™ with Heated Electrospray Ionization (HESI) ionization source and an Agilent® Technologies 6538 UHD Accurate-Mass quadrupole Time-Of-Flight (qTOF) with a dual Electrospray Ionization (ESI) source.

Wichitnithad et al.<sup>1</sup> have shown that when the protonated precursor ion of fentanyl at  $m/z$  337 is exposed to collisional activation, two distinct constitutional isomers of the product ion at  $m/z$  188 are formed. The two constitutional isomers are formed via the intermediate at  $m/z$  281. However,  $MS^n$  experiments in this work demonstrate another isobar at nominal mass  $m/z$  188 that has a distinct elemental composition from the other structures based on accurate mass measurements. This third ion forms via the intermediate fragment at  $m/z$  216 and is of interest because the abundance of  $m/z$  188 is commonly used for quantification with Multiple Reaction Monitoring (MRM)<sup>1</sup>, and one should be confident in the ions' identity and relative abundance.

$MS^n$  data on the linear ion trap mass spectrometer reveals that there are three pathways of formation for the nominal mass of  $m/z$  188, but that the isolation and  $MS^n$  fragmentation of  $m/z$  188 from different precursors provides distinct fragment ions. For example, the pathway  $m/z$  337→281→188→ provides a range of product ions consistent with the two distinct isomers at  $m/z$  188 that were first described by Wichitnithad et al.<sup>1</sup>. However, the current work shows that the pathway  $m/z$  337→216→188→ results in an exclusive fragment at  $m/z$  132 and that fragmentation through the pathway  $m/z$  337→244→188→ results in fragments at  $m/z$  134 and  $m/z$  120.

The proposed mechanism for the formation of the novel isobaric product ion involves the opening of the piperidine ring from the  $[M+H]^+$  fentanyl precursor followed by a 4-center-elimination of the saturated alkyl chain. The combination of  $MS^n$ , accurate mass measurements with HRMS, isotopic labeling, and differences in the  $MS^3$  product ion spectra allow for the identification of this novel fentanyl product ion and an enhanced understanding of the fragmentation behavior of fentanyl and FRCs.

*This project was supported by a grant awarded by the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the Department of Justice.*

### Reference(s):

- <sup>1</sup> Wisut Wichitnithad, Terence J. McManus, Patrick. S. Callery. Identification of isobaric product ions in electrospray ionization mass spectra of fentanyl using multistage mass spectrometry and deuterium labeling. *Rapid Commun. Mass Spectrom.*, 2010. 24(17): pp. 2547-53.

### Seized Drugs, Mass Spectrometry, Isotopic Labeling



### **B189 The Detection and Identification of Synthetic Cannabinoids by Portable Nanoflow Liquid Chromatography-Ultraviolet (LC-UV) Detection**

*David C. Pavone, BS\*, Washington, DC 20008-3413; Marisa C. May, BS, Alexandria, VA 22304; Ira S. Lurie, PhD, George Washington University, Washington, DC 20007*

**Learning Overview:** After attending this presentation, attendees will understand how synthetic cannabinoids can be screened for and/or positively identified using portable nanoflow LC-UV detection instrumentation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the first truly portable method of nanoflow LC with UV detection as a method for reliable detection and identification of synthetic cannabinoids. This methodology is not only applicable for field testing of these emerging drugs but presents a relatively low-cost alternative (Category A tests not available) to meeting the Scientific Working Group for Seized Drugs (SWGDRUG) requirements for drug identification.

Within the United States, drug abuse has been at an all-time high and has no indication of slowing down. In fact, cases involving drug overdose deaths have more than tripled since 1990. Many different factors have contributed to this rise in drug abuse, one of these being a sharp and steady increase of “emerging” drugs. These emerging drugs are defined as the variances of controlled substance analogs that are synthesized to circumvent existing drug laws. For this study, the type of emerging drug being investigated is synthetic cannabinoids. Synthetic cannabinoids are defined by being able to affect the same receptors in the brain as  $\Delta$ -9 Tetrahydrocannabinol ( $\Delta$ 9-THC). Commonly known as “Spice” or “K2,” synthetic cannabinoids are used as an alternative to marijuana because of their similar psychoactive effects. General drug screening techniques struggle to identify synthetic cannabinoids, compounded by the multitude of analogs that are continuing to be made every day.

As a complementary separation technique to Gas Chromatography (GC), various forms of LC have emerged for drug analysis. These forms of LC not only can be applicable to most if not all drugs, without the need for liquid-liquid extraction and/or derivatization, but also they can increase the reliability of retention time measurements to assist in compound identification. Ultraviolet (UV) detection, a complementary technique to electron ionization mass spectrometric detection for GC, is commonly employed for LC.

For field test purposes, the most common methods for synthetic cannabinoids include color and spectroscopic techniques. Color tests are highly susceptible to false positives. Other techniques, such as portable Raman and Fourier Transform Infrared (FTIR) spectroscopy, have proven to be effective in screening for drugs at remote sites, but struggle when trying to perform the analysis of mixtures. Not only is a portable nanoflow LC able to be used at remote sites, but it has also proven to be a reliable method for screening and/or identification of drugs in a mixture.

This presentation describes the use of a portable nanoflow LC instrument, which provides for specificity in detection of synthetic cannabinoids, applicable to mixtures. Drug detection is accomplished by employing tandem capillary columns in series with dual UV wavelength on-column detection, resulting in very low solvent usage, all in an instrument that weighs only about 16 pounds. The instrument generates dual uncorrelated retention times and peak area or peak height ratios, which are proportional to a compound’s extinction coefficient at both wavelengths. Two capillary columns in series (e.g., C8 and biphenyl) and dual UV LED detectors (e.g., 255nm and 275nm) are employed. Specificity in analysis arises from the unique ratios of dual relative retention times and peak area or peak height ratios from dual UV wavelength detection. The applicability for the screening and/or identification of these emerging drugs in various plant material matrices is presented. In regard to drug identification, the proposed instrumentation has the capability of adhering to the SWGDRUG guidelines by providing laboratories for which a Category A test is not available with two Category B tests (dual complementary retention times) and at least one Category C test (dual UV wavelength detection).

#### **Portable Nanoflow LC, Emerging Drugs, Synthetic Cannabinoids**





### **B190 2020 Update From the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)**

*Sandra E. Rodriguez-Cruz, PhD\*, Drug Enforcement Administration, Dulles, VA 20166*

**Learning Overview:** The goal of this presentation is to provide attendees with a summary of the most recent activities and work products developed by SWGDRUG.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing and discussing information and resources for the analysis of seized drugs.

SWGDRUG was formed in 1997 in a joint effort between the United States Drug Enforcement Administration (DEA) Office of Forensic Sciences and the Office of National Drug Control Policy (ONDCP). SWGDRUG works to improve the quality of the forensic examination of seized drugs and to respond to the needs of the forensic community by supporting the development of internationally accepted minimum standards, identifying best practices within the international community, and providing resources to help laboratories meet these standards. This presentation will provide attendees with information on SWGDRUG activities during the past year.

During the summer of 2019, core committee members approved the most recent version of the SWGDRUG Recommendations, version 8.0, which includes revisions to PART IIIB, Methods of Analysis/Drug Identification. A reliable and scientifically supported identification of a drug or chemical depends on the use of an appropriate analytical scheme by competent analysts in a quality-controlled process. The purpose of PART III B is to recommend minimum requirements for the forensic identification of such materials. PART III B addresses the overall selection of techniques, the rationale behind their categorization, and emphasizes the need to develop robust analytical schemes dependent on the scenario at hand or jurisdictional application.

A new supplemental document, SD-7 (Construction of an Analytical Scheme), was also approved during 2019. The objective of this supplemental document is to provide guidance to practitioners on the design and implementation of appropriate analytical schemes, as required by SWGDRUG Recommendations PART IIIB. It includes more than a dozen examples of analytical schemes applicable to many jurisdictions. During this presentation, some of these examples, their rationale, limitations, and applicability will be discussed.

SWGDRUG committee members are also working on revisions to PART IVA (Quality Assurance/General Practices) and PART IVB (Quality Assurance/Validation of Analytical Methods) of the Recommendations. Revisions include additional background information and clarifications on the performance characteristics to be evaluated during the validation of both qualitative and quantitative methods. Furthermore, the currently existing Supplemental Document SD-2 (Validation of Analytical Methods) is also being revised and expanded to better assist seized-drug practitioners during method validation activities. Additions include examples of how to perform and document validations for a color test, a Gas Chromatography/Mass Spectrometry (GC/MS) method, and an Infrared (IR) spectroscopy method.

This presentation will also summarize recent updates on multiple SWGDRUG resources, such as the MS library, IR library, and Drug Monographs.

The SWGDRUG core committee includes representatives from regional, national, and international forensic organizations; educators, practitioner and scientists from the United States; and representatives from the European Network of Forensic Science Institutes (ENFSI), the Academia Iberoamericana de Criminalística y Estudios Forenses (AICEF), the Asian Forensic Science Network (AFSN), and the United Nations Office on Drugs and Crime (UNODC).

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### **Criminalistics, Drug Analysis, SWGDRUG**



### **B191 Qualitative and Quantitative Characterization of Complex Samples Containing Fentanyl From Supervised Consumption Sites Using Quantitative Nuclear Magnetic Resonance (qNMR) and a Multi-Component Multiple Resonance Post-Processing Quantitative Algorithm**

*Richard R. Laing, MS\*, Health Canada, Burnaby, BC V5G 4P2, CANADA*

**Learning Overview:** After attending this presentation, attendees will be familiar with the capabilities of the NMRQUANT algorithm and quantitative and qualitative trends derived from more than 600 samples collected at supervised consumption sites in British Columbia, Canada, as part of a “Drug Checking” project.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on a new and innovative method for multi-component analysis allowing for complete characterization of street drugs using qNMR and a post-processing algorithm. Every component of a street drug mixture can be simultaneously quantitated, providing they possess a proton (1H) and are soluble in the analysis solvent (Deuterium Oxide [D<sub>2</sub>O]). Complex Controlled Substance (CS) mixtures are routinely characterized using this technique to provide trends of diverse mixtures, including diluents and adulterants. These trends will be presented along with the typical opioids and other CS mixtures found in supervised consumption sites in British Columbia, Canada.

According to the United States Centers for Disease Control and Prevention, 70,237 drug Overdose (OD) deaths occurred in the United States in 2017 of which 47,600, or 67.8%, involved a synthetic opioid.<sup>1</sup> The highest rate of OD deaths in 2016 was observed in West Virginia at >50 deaths per 100,000 population. Unfortunately, this trend is not unique to the United States. In Canada, on April 14, 2016, the province of British Columbia proclaimed a “Public Health Emergency” to alert policy makers, the public, and the media as to the impact synthetic opioids were having on local communities. In 2016, a total of 668 OD deaths, in which fentanyl was detected, rose to 1,229 in 2017 and to 1,334 in 2018 for all of British Columbia.<sup>2</sup> In the city of Vancouver, these annual figures rose from 152 to 306 to 337, respectively, in the same period.<sup>3</sup> To put this into perspective, for Vancouver in 2018, the OD death rate in which fentanyl was detected was 53 per 100,000, a number similar to West Virginia; however, this was in a small area of only 115km<sup>2</sup>.

In response to this crisis, all levels of government have contributed to numerous initiatives. One initiative, based on harm-reduction principals, is the extension of access to drug checking at supervised consumption sites where users can have their drugs tested for fentanyl using Fourier Transform Infrared (FTIR) and an immunoassay test strip prior to use. The premise of drug checking is that with the provision of knowledge of the drug composition, the user can make an informed decision about its usage. Because of Canada’s Federal Action on Opioids, Health Canada’s Drug Analysis Service (DAS), a network of forensic drug analysis laboratories, took on new activities in support of harm-reduction initiatives for health authorities and Non-Governmental Organizations (NGO). In 2018, DAS, through its Vancouver lab, piloted a project where “drug checked” samples could be submitted and undergo more rigorous testing. Using a 400MHz Bruker® Ascend™ Nuclear Magnetic Resonance (NMR) equipped with a CryoProbe™ Prodigy, the NMRQUANT algorithm can identify and quantitate more than 30 controlled substances and common adulterants or diluents in a 10mg sample to the 1% level. The algorithm, based on the programming language Julia, uses a chemical shift and integral library calculations against the response of an internal standard so that individual components of a mixture can be identified and quantified during the same experiment without the requirement for chromatographic separation. As of August 2019, approximately 600 samples have been submitted for analysis where the principal components have been quantitated, size permitting. Approximately 70% of all samples contained fentanyl at varying concentrations. Fentanyl was found as the primary, secondary, or tertiary controlled substance, and common mixtures of heroin and fentanyl or fentanyl and methamphetamine have been characterized as well. Nearly 80% of all samples contain caffeine, with mannitol being the preferred cutting agent. An overview of the qNMR experiment, NMRQUANT method, and trend results will be presented and discussed.

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2. *Fentanyl-Detected Illicit Drug Toxicity Deaths January 1, 2012 to May 31, 2019*. [Internet]. 2019. Available from: <https://www2.gov.bc.ca/assets/gov/birth-adoption-death-marriage-and-divorce/deaths/coroners-service/statistical/fentanyl-detected-overdose.pdf>.
3. *Vancouver’s approach to the Overdose Crisis*. [Internet]. 2019. Available from: <https://vancouver.ca/people-programs/drugs.aspx>.

#### **Fentanyl, qNMR, Multi-Component Analysis**



### B192 Examination of Fentanyl and Fentanyl Analog Samples in Canada: Trends and Strategies for Analysis

*Michelle Boileau, PhD\*, Drug Analysis Service, Health Canada, Toronto, ON M1P 4R7, CANADA*

**Learning Overview:** After attending this presentation, attendees will understand the trends in fentanyl and fentanyl analog samples submitted for analysis to the Drug Analysis Service Laboratories over the past several years. Additionally, attendees will be introduced to liquid chromatography-based methods for the identification and quantitation of fentanyl and fentanyl analogs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the recent trends of fentanyl and fentanyl analog samples submitted for analysis in Canada. Additionally, this presentation will impact the forensic science community by providing an introduction to Liquid Chromatography coupled with Triple Quadrupole Mass Spectrometry (LC/TQMS) and Liquid Chromatography coupled with quadrupole Time Of Flight Mass Spectrometry (LC-qTOF/MS) for the identification and quantitation of fentanyl and fentanyl analog samples.

Fentanyl and fentanyl analogs have become a major concern in Canada. Since 2012, there has been an almost 4,100% increase in the number of fentanyl and fentanyl analog samples being submitted to the Drug Analysis Service Laboratories for analysis. Not only has the number of samples increased, but the composition and type of samples have also changed dramatically over this same time period. The changes in sample type and composition have necessitated a change in the type of analysis performed. The fentanyl and fentanyl analog samples tend to be low-dose/high-potency samples and traditional methods of analysis, such as Gas Chromatography coupled with Flame Ionization Detector (GC/FID) and Gas Chromatography coupled with Mass Spectrometry (GC/MS), may not be sufficient on their own to identify these compounds. As a result, the Drug Analysis Service Laboratories have incorporated the use of LC/TQMS and LC/qTOF for the identification and quantitation of these samples.

The low concentration of the fentanyl and/or fentanyl analogs present in the samples submitted provided challenges to their analysis. The increase in sample preparation concentration needed to obtain a positive result also increased the risk to the analysts themselves during sample handling. The high sensitivity of the LC/TQMS allowed for lower concentration samples to be effectively analyzed, thereby reducing the risk to the analyst during sample preparation. The new LC/MS/MS method allowed for the identification of 27 fentanyl and fentanyl analogs. These compounds are fentanyl, acetylfentanyl, butyrylfentanyl,  $\beta$ -hydroxythiofentanyl, furanylfentanyl, carfentanil, 4-ANPP, meta-fluorofentanyl, para-fluoroisobutyrylfentanyl, para-fluorobutyrylfentanyl, alpha-methylfentanyl, isobutyrylfentanyl, cis-3-methylfentanyl, trans-3-methylfentanyl, W-18, cyclopropyl fentanyl, crotonyl fentanyl, methoxyacetylfentanyl, acrylfentanyl, para-chlorofentanyl, para-chloroisobutyrylfentanyl, 4-methoxybutyrylfentanyl, ocfentanil, valerylfentanyl, alpha-methylbutyrylfentanyl, W-15, and U-47700.

As a result of the high volume of quantitation requests, discussed below, a method was developed using LC/qTOF to assist the LC/TQMS with the identification of these types of samples. The method developed on the LC/qTOF is able to identify approximately 36 different fentanyl and fentanyl analogs. With the exception of 4-ANPP, this method is able to identify all the fentanyl compounds identified by the LC/TQMS identification method plus 4-APP, benzoylfentanyl, benzylacrylfentanyl, benzylcarfentanil, benzylfentanyl, ortho-methylfuranylfentanyl, para-chlorofuranylfentanyl, U-48800, U-49900, and U-5174.

Due to the increase in overdose deaths, more and more law enforcement agencies inquired as to whether it would be possible to determine the concentration or amount of fentanyl and/or fentanyl analog present in the samples submitted for analysis. Consequently, a quantitation method was developed on the LC/TQMS that is currently capable of quantifying five different fentanyl and fentanyl analogs. These compounds include fentanyl, acetylfentanyl, carfentanil, furanylfentanyl, and cis-3-methylfentanyl. The five compounds selected for inclusion in this method were based on the samples submitted for analysis. Additional fentanyl analogs can be added should the need arise.

The nature of the samples submitted to the Drug Analysis Service Laboratories for analysis have changed over the years. This is especially true for those samples containing fentanyl-related compounds. The high sensitivity of LC/TQMS and LC/qTOF provides the forensic drug chemist additional tools in the identification and quantitation of these types of samples.

#### **Fentanyl, LC/TQMS, LC-qTOF**



### **B193 Using Silica-Hydrate-Based Stationary Phases for Dual-Mode Ultra High Performance Liquid Chromatography (UHPLC) Separation of Synthetic Cathinone Positional Isomers**

*Carly Ploumen, BS\*, Alexandria, VA 22302; Ioan Marginean, PhD, The George Washington University, Washington, DC 20007; Ira S. Lurie, PhD, The George Washington University, Washington, DC 20007*

**Learning Overview:** After attending this presentation, attendees will understand the great utility of silica-hydrate-based LC columns for the separation of synthetic cathinone positional isomers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing methodology that will enhance the separation power of traditional LC technology employed for the separation of positional isomers of emerging drugs by involving the use of silica-hydrate-based stationary phases, allowing for more accurate identification of these compounds.

Synthetic cathinones are a class of “emerging drug” commonly referred to and marketed as “bath salts.” Synthetic cathinones provide users similar psychostimulant and hallucinogenic effects as amphetamine, cocaine, and MDMA, but at a much cheaper price and with possibly lower legal liability. Like many other classes of emerging drugs (fentanyl, synthetic cannabinoids, and phenethylamines), many positional isomeric forms of synthetic cathinones exist, making identification of these drugs difficult for forensic laboratories.

Tandem techniques, such as Gas Chromatography/Mass Spectrometry (GC/MS) and LC/MS are employed for the analysis of emerging drugs. The identification of positional isomers using MS detection can be difficult, especially when substitution occurs on the benzene ring. Photo Diode Array Ultraviolet (PDA UV), vacuum-UV, and Fourier Transform Infrared (FTIR) detection for GC and LC have proved to be capable of distinguishing between some or all isomeric forms of these compounds. Due to the high degree of structural similarity between positional isomers, separation is often difficult. GC is commonly employed by laboratories to analyze synthetic cathinones, but derivatization and/or liquid-liquid extraction is normally required. LC (either characterized as High-Performance Liquid Chromatography [HPLC] or UHPLC) uses Reversed Phase Chromatography (RPC) or Hydrophilic Interaction Chromatography (HILIC) that allows for the direct analysis of these compounds. Due to the presence of a water layer formed during the chromatographic process, equilibration and repeatability in HILIC is challenging.

For Silica-Hydrate (SiH) -based stationary phases, under Aqueous Normal Phase (ANP) conditions, a negligible water layer exists surrounding the stationary phase. ANP chromatography, similar to HILIC, employs an aqueous mobile phase with high organic content. Unlike HILIC, equilibration times are shorter and run times exhibit good repeatability. Another favorable aspect of SiH columns is that they can be operated under both RP and ANP modes. This dual-mode capability is highly advantageous in that a single sample can be run under both chromatographic modes using the same solvent reservoir solutions without the need to change columns. This methodology yields orthogonal retention times and dual UV wavelength detection, thereby significantly increasing the reliability of compound identification.

A mixture of eight synthetic cathinone positional isomers was analyzed by UHPLC-PDA/MS. Several SiH columns (C18, Phenyl Hydride, UDC Cholesterol, Diamond Hydride, and Silica-C) were run under RP and ANP conditions. Results were compared to the use of classical RPC and HILIC columns: C18, Silica, and Pentafluorophenyl (PFP). The PFP column also exhibits dual mode capability. “Optimum” separation conditions were determined for each column, and column selectivity, reversibility, and repeatability were explored.

Significant selectivity differences were observed using bi-modal columns and between different stationary phases. The SiH Silica-C column, which contains no derivatized ligands attached to the silica-hydrate backbone, not only gave the most orthogonal separations of the bi-modal columns, but provided a unique separation of all 8 positional isomers (resolution>1) using ANP. Although the combination of RPC and HILIC columns gave the most orthogonal separations (with slightly greater selectivity than Silica-C stationary phase), allowing for a separation of all the positional isomers, poor repeatability was obtained using HILIC. In contrast, the Silica-C column, under ANP conditions, produced great separation and repeatability. The ability to use a single column to perform two types of orthogonal chromatographic separations with good repeatability would be beneficial for the separation of positional isomers of emerging drugs.

#### **Silica-Hydrate Stationary Phases, Emerging Drugs, Positional Isomers**



### **B194 An Analysis of Emerging Benzodiazepines by Thermal Desorption/Direct Analysis in Real Time-Mass Spectrometry (TD/DART®-MS)**

*Sydney Jones, BS\*, Baltimore, MD 21209; Edward Sisco, PhD, NIST, Gaithersburg, MD 20899; Ioan Marginean, PhD, The George Washington University, Washington, DC 20007*

**Learning Overview:** After attending this presentation, attendees will better understand the capabilities of TD/DART®-MS to detect and identify benzodiazepines in pure form as well as in binary mixtures, complex matrices, and real case samples. Attendees will also gain an understanding of the capabilities and weaknesses of this technology and sampling strategies for presumptive screening.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an optimized method for the use of an emerging technology, TD/DART®-MS, for the detection and analysis of the rising number of benzodiazepine analogs.

Novel Psychoactive Substances (NPSs) represent a prominent and increasing concern for forensic laboratories across the country. Benzodiazepines belong to one class of NPSs that has seen a growing presence in the United States. The rise of benzodiazepines, driven predominantly through the increased production and prevalence of counterfeit pharmaceutical tablets, may lead to another drug epidemic. The development of new techniques, or new methods for existing techniques, allowing for rapid detection and identification of these compounds is required for laboratories to rapidly respond to this emerging concern.

There is an increasing need for rapid, reproducible, and precise identification of NPSs such as benzodiazepines to assist presumptive and confirmatory analyses. As drug units across the country are facing overwhelming backlogs, the ability to rapidly characterize a drug sample, from a field collection, a swipe, or an extract, with a highly specific and reproducible screening technique could provide substantial progress in both reducing case backlog and lowering turnaround time. TD/DART®-MS was investigated in this work as a technology that can face these challenges. TD/DART®-MS, which is a modification of DART®-MS that incorporates a thermal desorption unit, has been shown to provide increased reproducibility, sensitivity, and analyst safety compared to traditional analyses. This modification also allows for analysis via swipes, instead of glass capillary tubes, which has been demonstrated to be an effective way to screen evidence or evidence packaging. This project aimed to develop and understand the capabilities to detect benzodiazepines using TD/DART®-MS.

The initial component of this study used a design of experiments approach to determine the key instrumental parameters and led to the creation of an optimized method. Parameters that were optimized included: DART® ionization gas, Vapor flow rate, thermal desorber temperature, and DART® exit grid voltage. The optimized method was then used to collect representative spectra and create a library at different fragmentation voltages for a suite of 19 different benzodiazepines. Sensitivity of the method was then determined by calculating the limit of detection for a select number of compounds and was found to be on the order of single nanograms per swipe. Studies were also completed to identify the effect common cutting agents, such as stearic acid, mannitol, lactose, and caffeine, and additional drugs, such as heroin, had on the ability to detect the benzodiazepines in mixtures. Additionally, the incorporation of three complex background matrices for swipe-based collection (fingerprints, dirt, and plasticizers) to the samples was completed to test potential ion suppression. Real case samples were also analyzed to demonstrate the utility of the technique for a practicing forensic laboratory.

This work demonstrates that rapid detection of benzodiazepines with nanogram sensitivity is achievable using TD/DART®-MS with minimal issues caused by complex matrixes and mixtures. The detection of low sample quantities can be advantageous to forensic scientists as it provides a sensitive and rapid analysis of target analytes using swipes of evidence packaging, evidence itself, or extracts.

#### **Drug Analysis, TD/DART®-MS, Benzodiazepines**



### **B195 A Comparison of Expected Drug and Confirmed Findings in Samples Submitted by Clients of Supervised Consumption Sites in British Columbia**

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**Learning Overview:** After attending this presentation, attendees will understand the nature of the illicit drug supply in British Columbia, Canada, as it pertains to adulteration, contamination, and misrepresentation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing some common misconceptions about the nature of the illicit drug supply

**Background/Introduction:** Opioid-related deaths have increased dramatically in the past five years in British Columbia, which have mainly been driven by fentanyl and its analogs in the illicit drug supply. While there is speculation that fentanyl can be present in all types of illicit drugs, there is a paucity of literature that directly compares the expected drugs that are purchased illicitly with confirmed drug identification. Counterfeit tablets that resemble pharmaceutical opioids or benzodiazepines have been adulterated with fentanyl. Clinical and postmortem testing of biological samples often show co-occurrences of fentanyl and stimulants, which has led to speculation that stimulant drugs may also be adulterated with fentanyl. Identification of drug samples seized by border and police services in British Columbia has shown a co-occurrence of opioids and stimulants (such as cocaine and methamphetamine) of approximately 10%. However, it is not clear if seized drugs and toxicology results are representative of the local illicit drug supply. A better understanding of the drug supply is needed in order for policy makers, health care professionals, and harm reduction workers to assist in providing a less risky environment for people who use illicit drugs.

**Objective:** To characterize the illicit drug supply by comparing expected drug identity with results obtained from laboratory analysis.

**Method:** Clients at select supervised consumption sites in British Columbia, Canada, who utilize on-site drug checking services provided staff with information on the expected identity of their purchased street drug and approximately 5mg of sample. Powdered samples were submitted to the Provincial Toxicology Centre for analysis using Gas Chromatography with Mass Spectrometry (GC/MS) in full scan mode and compared to commercial libraries and in-house verified standards.

**Results:** In total, 223 samples were submitted for this study; 163 (73%) were primarily expected to be opioids, and 31 (14%) were expected to be cocaine or methamphetamine; the remaining 29 (13%) samples were not expected to contain opioids, cocaine, or methamphetamine. Among all expected opioids, 125 (77%) had fentanyl detected, 20 (12%) had methamphetamine detected, and 7 (4%) had cocaine detected. Fentanyl was the expected substance in 48% of submitted samples. Of those expected to contain fentanyl by clients ( $n=107$ ), 83% contained fentanyl. Expected fentanyl samples that did not contain fentanyl had at least one of: diacetylmorphine (i.e., heroin), a fentanyl analog, or a synthetic cannabinoid. One expected fentanyl sample contained only methamphetamine. Heroin was expected in 23% of submitted drugs. Among the 30 samples that were purchased as heroin, 73% had heroin detected and 50% had fentanyl detected. Expected stimulants were generally in good agreement with detected substances. However, two expected cocaine samples contained fentanyl but did not contain any stimulants. Of the five counterfeit “Xanax” tablets that were submitted, none contained alprazolam but did contain other central nervous system depressants, including fentanyl analogs, etizolam, and antihistamines.

**Conclusion/Discussion:** Illicit opioids and benzodiazepines often contain fentanyl or its analogs, regardless of expected substance. Stimulants are generally not adulterated with opioids or other depressants. However, two cases were identified in which fentanyl but not cocaine was present in expected cocaine samples. This may be due to a number of reasons, including mislabeling, accidental misclassification, or intentional adulteration. Efforts to increase sample size for expected stimulants are underway.

#### **Drug Identification, GC/MS, Illicit Drug Supply**



### **B196 Identifying Powdered Illicit Drugs Using Magneto-Archimedes Levitation (MagLev), Signal Processing, and Digital Finger Printing**

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**Learning Overview:** After attending this presentation, attendees will have learned how MagLev can be used to separate and determine the composition of powdered mixtures. This presentation will focus on how signal processing of the MagLev data is used to generate digital fingerprints that are used for automated identification and attribution of illicit drugs (e.g., fentanyl and its analogs, heroin, methamphetamine, and cocaine) in powdered mixtures with adulterants and diluents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how the use of density-based analysis techniques and signal processing methods can simplify and accelerate the analysis of complex mixtures found in forensics. The digitalization of the MagLev data enables comparison with previously characterized samples, and this could aid forensic chemists in achieving attribution of their samples.

**Methods:** Dry and powdered samples are suspended in a solution of a non-polar paramagnetic gadolinium(III) chelate in a non-polar solvent inside a cuvette (polar compounds do not dissolve as they separate in the paramagnetic solution). The cuvette is placed between two magnets with like poles facing each other. The balance of forces (magnetic, buoyancy, and gravity) form a linear density gradient along the vertical axis of the cuvette, in which the individual components of the powder equilibrate at their characteristic density, forming a levitation profile. By photography, image analysis and digital classification (Dynamic Time Warping [DTW] and DTW Barycenter Averaging), the sample is automatically attributed to a class (a digital fingerprint), such as mixtures of “lidocaine-HCl” or “caffeine,” and entered into a database of class representations of mixtures of powdered drugs. Unknown mixtures are compared to the class representations to determine their maximum likelihood-classification.<sup>1</sup>

**Results:** MagLev and signal processing techniques were applied to three problems relevant to the testing of illicit drugs: (1) identification of individual components (fractions) in powdered mixtures using automated signal processing of levitation profiles. In addition, classification techniques are used for the identification of components in mixtures of unknown composition (e.g., an unknown fraction of lidocaine HCl is identified in a powdered mixture from multiple different class representations, including fentanyl-HCl, heroin-HCl, acetaminophen, levamisole HCl, lactose, mannitol, and sucrose); (2) the abundance of lidocaine-HCl and caffeine in binary mixtures of different compositions were determined; and (3) classification techniques were used for the attribution of powdered samples identifying potential matches (batch and formulation) between previously characterized samples in a laboratory database.

**Conclusions:** MagLev is a rapid and portable method for screening of drugs. It separates mixtures into their individual components and reduces the time for identification of illicit drugs. This method establishes and uses a database of digital fingerprints of powdered mixtures of illicit drugs. In the future, the database could be combined with data about the point of seizure and a time stamp map drug trafficking patterns. The device and analysis methods are adaptable to other applications, such as powdered chemicals, food products, or explosives.

#### **Reference(s):**

- <sup>1</sup> Nemitz, Markus P., Ryan J. Marcotte, Mohammed E. Sayed, Gonzalo Ferrer, Alfred O. Hero, Edwin B. Olson, and Adam A. Stokes. Multi-Functional Sensing for Swarm Robots Using Time Sequence Classification: Hoverbot, an Example. *Frontiers in Robotics and AI* 5 (2018): 55.

#### **Magneto-Archimedes Levitation, Drugs, Signal Processing**



## B197 Machine-Learning (ML) Approaches for Source Attribution of Forensic-Relevant Materials

Josh Dettman, PhD\*, Massachusetts Institute of Technology Lincoln Laboratory, Lexington, MA 02420; Amanda M. Casale, Massachusetts Institute of Technology Lincoln Laboratory, Lexington, MA 02421

**Learning Overview:** After attending this presentation, attendees will better understand how Artificial Intelligence (AI) ML algorithms are enabling new applications requiring the classification of large data sets, including extracting conclusions on the source of materials using chemical and physical signature data.

**Impact on the Forensic Science Community:** Applications of AI and ML techniques are growing rapidly, including in forensic science. This presentation will impact the forensic science community by increasing competence in ML by introducing potential ML approaches and disseminating methods for material source attribution to the operational community and other researchers.

Source attribution of materials involved in a crime using chemical and physical signatures can be an important technique for generating investigative leads on the origination location (source) of the material and potential association with a suspect. Proof-of-concept studies of this type have been performed for a variety of materials of forensic and commercial importance. Chemometric or, more generally, ML techniques can be used to extract conclusions from the relatively high-dimensional data collected and estimate the probability that a sample originated from a specific source. This presentation will describe the development of a composite ML sourcing algorithm utilizing multiple tiers of supervised ML classification methods, data conditioning, bootstrap supplementation of reference data, and probability-weighted results fusion to estimate the probability of source membership of fertilizer samples based on chemical and physical signature data.

Reference samples of fertilizer were obtained from known commercial and industrial sources. For most sources, only single samples were available (single-lot sources); however, nine to ten samples were collected over a period of five months from two sources (multi-lot sources) to estimate signature variance over time. After evaluation of many potential signature types, the final set of signatures collected from the reference samples was comprised of reflectance spectra (color), particle morphology (size/shape), and trace element composition. This data was subjected to limited conditioning (e.g., conversion from intensity to element concentration by a calibration curve, calculation of color, and shape summary variables). Additional bootstrapped reference data for algorithm training was performed based on the signature means (by source) and largest experimentally measured variance for each signature (by source and lot). Increasing the signature variance above what was empirically measured incorporates an estimate of source variance (not otherwise possible with the largely single-lot source sample set) and provides a more conservative and, notionally, realistic estimate of operational classification performance.

The relative probability that the unknown sample arose from one of the reference sources (or from a previously measured unknown source) is determined using a composite ML algorithm consisting of three parts: (1) elimination of the least likely sources using a  $k$ -Nearest Neighbors ( $k$ -NN) classifier for each of the three signature types; (2) a Multinomial logistic Regression (MNR) classifier to aid the examiner in assessing if a reference sample matching the unknown sample is present in the reference database or not; and (3) three random forest classifiers (one for each signature type) for final estimates of the relative match probability for the unknown sample to each of the remaining sources from the reference database. The source probabilities from the random forest classifier for each signature type are weighted by  $z$ -score across all of the source probabilities, then averaged and normalized to give “fusion scores” that are relative weighted probabilities. The individual signature and fused relative probabilities, as well as the raw signature data, are presented to the examiner in a user-focused web interface for further analysis and a source assignment decision.

To estimate the algorithm performance conservatively and as realistically as possible given inherent sample collection limitations, cross-validation testing was done by holding out signature data from entire lots (rather than a random subset of single samples) during training and using the entire held-out lots for subsequent testing. For the samples from 23 fertilizer sources that were tested, the correct source is chosen in cross-validation as the most likely source 87% of the time and is an average of 2.3 times as likely as the most probable incorrect source. These relatively accurate and confident sourcing results, even under conservative performance estimate conditions, indicate the promise of the signatures and composite ML sourcing algorithm for determining the source of unknown fertilizer samples.

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### Machine Learning, Artificial Intelligence, Attribution





### B198 A Regression-Based Algorithm to Maximize the Confidence in Mass Spectral Identifications

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**Learning Overview:** After attending this presentation, attendees will have learned the major sources of random and non-random variance in mass spectrometric analyses and the value of ion correlation analysis. Attendees will also know how to use the correlation that exists between ion abundances in replicate spectra to make compound identifications that are more confident and more accurate than existing algorithms.

**Impact on the Forensic Science Community:** This presentation will provide the forensic science community with a mathematical model for compound identification from mass spectrometric data that is more accurate and more precise than current static/discrete methods. The application of a more selective algorithm will decrease the incidence of false positives and further assist with the identification of unknown compounds in casework.

**Hypothesis:** It was hypothesized that a mathematical model that takes into account the covariance between ion abundances will provide better discrimination between true positives and true negatives than an algorithm that does not take into account the covariance between ion abundances, and for the model to work, several other hypotheses about the data must be also be true, one of which is the expectation that the residuals in the predictions should be normality distributed.

**Methods/Results:** Current mass spectrometric methods of substance identification use a “static” algorithm to determine the identity of a substance. The “static” approach assumes that there is one “best” or average exemplar of a substance in a library against which unknowns are compared. However, the variance in ion abundances for replicate spectra is around  $\pm 20\%$  (95% Confidence Level [CL]), which can result in false positives in substance identifications. Instead, the algorithm uses a multivariate general regression model between ion abundances to make ion abundance predictions within a measured spectrum.

A mixture containing five drug standards was analyzed several times a day for approximately two weeks. From the approximate two dozen Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS) data files, hundreds of unaveraged mass spectra of each drug were extracted and split into a training set and an external validation set. The ion abundances were normalized to the base peak for each drug, and the 15 most abundant ions were selected iteratively to be the dependent variables within the general linear models. The model predicted ion abundances at each  $m/z$  value, and these predicted abundances were then compared to the measured spectra using Pearson Product-Moment Correlations (PPMCs) or Root Mean Squared Errors Of Predictions (RMSEP). The PPMC values for known positives and known negatives are then compared to a range of different threshold PPMC values to assess the True Positive (TP), True Negative (TN), False Positive (FP), and False Negative (FN) rate at each threshold. These assessments were used to construct Receiver-Operator Characteristic (ROC) curves, which provided Areas Under the Curve (AUC) of 1, or errorless classification. A separate test was conducted to assess the number of spectra necessary to create an accurate model for a certain compound. Varying numbers of spectra were used to create general linear models, and the PPMC values and Root Mean Square Errors (RMSEs) were compared.

Whereas the AUC of ROC curves for the prediction of external validation spectra was 1 for each drug model, the PPMC threshold at which no mistakes were made varied slightly for each drug. For example, diacetylmorphine and fentanyl required PPMC thresholds between  $\sim 0.5$ – $0.625$  to distinguish TPs from TNs, but cocaine, ecgonine methyl ester, and 6-monoacetylmorphine required PPMC thresholds of  $\sim 0.88$ – $0.95$  to achieve the same errorless identifications. When changing the number of spectra used to make a  $\Delta^9$ -tetrahydrocannabinol model, a model constructed from as few as 30 spectra performed just as well as a model built with 200 spectra.

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#### Dynamic Algorithm, Mass Spectrometry, Ion Correlations



### **B199 Detection and Differentiation of Derivatized Controlled Substances by Gas Chromatography-Vacuum Ultraviolet (GC-VUV) Spectrophotometry**

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**Learning Overview:** The goal of this presentation is to bring an understanding of GC-VUV spectrophotometry as applied to derivatized controlled substances.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the capabilities of GC-VUV spectrophotometry to detect and differentiate derivatized controlled substances.

GC-VUV is a technique with the ability to differentiate isomers based on their spectra and is complimentary to Mass Spectrometry (MS). By probing sigma bonds and high energy pi bonds in the region of light from 120–430nm, every molecule aside from diatomic hydrogen can be detected. As interest in the technique grows, questions arise such as how various derivatization reactions change the spectrum of a molecule. To answer this question, the change in spectra by silylation and acylation of drugs, including methamphetamine, methcathinone, ephedrine, pseudoephedrine, fentanyl, and carfentanil, were analyzed by GC-VUV.

Derivatization is performed when working with analytes having low volatility, thermal instability, or when functional groups known to adversely affect the chromatographic performance of the analyte are present. By exchanging polar or active groups with more non-polar or inactive groups, increased volatility and improved chromatographic performance on polydimethylsiloxane columns can be obtained. Derivatization also improves the thermal stability of compounds such as methcathinone, which is known to degrade with time, temperature, and light exposure. When analyzed by GC/MS, derivatized compounds experience a shift in retention time and fragment ions with increased mass based upon the added functional group(s). Using GC-VUV, the retention time shift is maintained, but the difference is seen in absorptivity. Acylating reagents such as Trifluoroacetic Anhydride (TFAA) add a new pi-bond to the molecule that is detected in the VUV spectrum, alkylating and silylating reagents will adjust the sigma-bond regions. Vibronic coupling of the molecules will result in altered spectra based on distance of the functional groups to VUV absorptive groups within the molecule. Compounds with no active hydrogens, such as fentanyl and several of its analogs, require a different derivatization reagent, such as Pentafluorobenzyl-Hydroxylamine (PFBHA), an acylation reagent, to react with the ketone group.

Results indicate improved response in the pi-bond region of the spectra for acylation derivatization of most compounds. Improved response leads to increased peak area, which produces lower limits of detection and quantitation than for non-derivatized forms of the same drug. Peak shape in the form of asymmetry also improves for compounds with active functional groups (i.e., methamphetamine). Several de-identified “street” samples were analyzed to show “real world” performance.

It should be noted that VUV has difficulties differentiating small alkanes and should be considered as complimentary to GC/MS rather than as a replacement. Overall, GC-VUV continues to show promise for future use in forensic drug analyses as a technique complimentary to GC/MS.

*This work was funded by a grant from the National Institute of Justice and does not necessarily reflect the opinions of the National Institute of Justice.*

#### **GC-VUV, Derivatization, VUV Detection**



### **B200 An Evaluation of Four Fentanyl Colorimetric Testing Options for Field Use**

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**Learning Overview:** After attending this presentation, attendees will be familiar with multiple, available, on-site colorimetric test kits for fentanyl and the most effective choice for field work in terms of accuracy. Attendees will see the challenges encountered when field testing fentanyl analogs and mixtures with colorimetric test kits.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a side-by-side comparison of four available fentanyl field test kits evaluating accuracy, reliability, and ease of use. Determining the best method for on-site fentanyl detection will allow for safer handling and securement of fentanyl for law enforcement personnel.

Over the past several years, the country has experienced a surge of opioid use, specifically a rise in the synthetic opioid, fentanyl. The rise of fentanyl use has led to many concerns. Most alarming are the dangers associated with its potency, as well as the risk of accidental exposure as the drug is a known inhalation and contact threat. These facts have led law enforcement personnel to improve on-site protocols looking for efficient, safe, and accurate field detection methods for the detection of fentanyl. The purpose of this research was to evaluate four colorimetric fentanyl field tests to determine the most accurate and reliable method for fentanyl field detection, taking into account ease of use for untrained personnel to easily evaluate a scene when unknown powders are present. Early detection could allow other law enforcement personnel responding to the incident to upgrade their Personal Protection Equipment (PPE) and keep the area contained for the safety of bystanders. Field detection of fentanyl could also contribute to safer handling, packaging, transporting, or shipping of evidence for analysis or court-related proceedings. Additionally, chemists or those further handling the evidence could be alerted to the potential risks of the exhibit.

This evaluation focused on the colorimetric tests' ability to detect known fentanyl and some of its common analogs and fentanyl in mixtures, such as in a mixture with heroin. One hundred case samples were evaluated using four field colorimetric tests: the Presumptive Drug Test (PDT) Fentanyl Reagent Box, the Narcotic Analysis Reagent Kit (NARK II), the MDT Fentanyl, and the MMC Fentanyl/Oxycodone, Heroin/Morphine, Codeine Test Kit. When assessing whether each of the four colorimetric tests would correctly identify the primary controlled substance present in each case sample, the NARK II correctly presumptively indicated fentanyl 77% of the time. As for correctly identifying fentanyl samples and mixtures containing fentanyl or fentanyl-related compounds, the NARK II colorimetric test presumptively indicated fentanyl correctly 50% of the time, while the PDT colorimetric test correctly presumptively indicated fentanyl 75% of the time. The results indicated that NARK II indicates heroin instead of fentanyl in mixtures where both heroin and fentanyl are present. While the PDT color test outperformed the NARK II in terms of fentanyl detection, the PDT test also had the most false positives than the other tests evaluated. Based on the colorimetric test results coupled with the preliminary observations, the NARK II colorimetric test had a higher presumptive indication rate for fentanyl/heroin identification in the field out of the four colorimetric tests evaluated.

#### **Heroin, Fentanyl, Color Tests**



### B201 Investigating the Robustness of a Statistical Method to Compare Mass Spectra of Fentanyl Analogs

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**Learning Overview:** After attending this presentation, attendees will be familiar with a method for the statistical comparison of mass spectra that can be used as a tool to increase confidence in identifications. Positional isomers of Fluorobutyryl Fentanyl (FBF) and Fluoroisobutyryl Fentanyl (FIBF) will be used to demonstrate application of the method, taking into account the effect of concentration and mass spectral tune conditions on the association and discrimination of the isomers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing a method by which two mass spectra can be compared statistically to increase confidence in controlled substance identification, which is especially useful for positional isomers and other structurally similar compounds.

The typical method for the identification of controlled substances is to analyze samples using Gas Chromatography/Mass Spectrometry (GC/MS) and to perform a visual comparison of the resulting mass spectrum to a suitable reference spectrum. However, for spectra of structurally similar compounds, visual comparison of spectra for identification can be challenging. Previous work in the Michigan State University laboratory focused on the development of a statistical method to compare the mass spectrum of an unknown sample to a suitable reference spectrum using an unequal variance *t*-test. In this approach, *t*-tests are used to statistically compare the mean abundances at every corresponding *m/z* value in the scan range for the two spectra at a user-defined confidence level. The null hypothesis ( $H_0$ ) being tested is that the difference between the mean abundances of a given *m/z* value is equal to zero. The alternative hypothesis ( $H_a$ ) states that the difference between the mean abundances of a given *m/z* value is not equal to zero. If  $H_0$  is accepted at each *m/z* value, the two spectra are statistically indistinguishable and a random-match probability is calculated to estimate the probability that the fragmentation pattern occurred by random chance alone. However, if  $H_a$  is accepted at any *m/z* value in the scan range, the two spectra are statistically distinguishable, and the ions responsible for discrimination are identified.

The statistical comparison method has been applied to differentiate amphetamine-type stimulants as well as positional isomers of ethylmethcathinone and fluoromethamphetamine. While the method has shown potential for differentiation of isomers, in the current work, the robustness of the method is tested by investigating the effect of analyte concentration and the effect of mass spectral tuning on the ability to associate and discriminate isomers.

In this work, GC/MS was used to analyze two sets of fentanyl isomers, which included the *ortho*-, *meta*-, and *para*- forms of FBF and the *ortho*-, *meta*-, and *para*- forms of FIBF. Compounds within each set were initially analyzed under equivalent conditions over several days, and the resulting spectra were statistically compared to assess association of corresponding isomers with discrimination from the others.

Initial results indicate the potential of the method for differentiation of these isomers. For example, spectra of corresponding isomers of FBF were statistically associated at the 99.9% confidence level with discrimination from the other FBF isomers. More specifically, *ortho*-FBF was discriminated from *para*-FBF and *meta*-FBF at the 99% confidence level with nine ions responsible for discrimination in each case, including common ions of *m/z* 44, 71, 102, 118, 144, 164, and 171. Similarly, *para*-FBF was discriminated from *meta*-FBF at the 99% confidence level, with three ions responsible for discrimination, *m/z* 44, 176, and 234. A long-term study is currently being conducted to investigate the effect of concentration and the effect of mass spectral tune conditions on the association and discrimination of these isomers.

In this presentation, the statistical method and ions responsible for discrimination will be described in more detail. Further, the results of the long-term concentration and tune studies will be presented to demonstrate the robustness of the method.

#### Mass Spectral Comparison, Fentanyl Analogs, GC/MS



### **B202 The Differentiation and Identification of Fentanyl Analogs Using Gas Chromatography Interfaced With an Infrared Detector (GC/IRD)**

*Agnes D. Winokur, MS, Drug Enforcement Agency, Southeast Laboratory, Miami, FL 33182; Lindsay M. Kaufman, BS\*, Drug Enforcement Administration, Miami, FL 33182; Jose R. Almirall, PhD, Florida International University, Miami, FL 33199*

**Learning Overview:** The goals of this presentation are for attendees to : (1) obtain a better understanding of the analytical challenges associated with fentanyl-related substances; and (2) obtain knowledge and understanding of how to apply GC/IRD methodologies for the separation and identification of fentanyl-related substances.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining how GC/IRD methodologies can be optimized to rapidly detect and identify structurally similar fentanyl-related substances in complex mixtures when conventional analytical techniques yield limited information.

The United States declared a national public health emergency in October of 2017 in response to the Opioids Crisis. Since 2014, there has been a significant increase of drug-related deaths attributed to the abuse of opioids, with more than 60,000 deaths in 2017. Fentanyl and related substances are among the opioids having the most impact on drug overdose deaths. Following the trend of fentanyl-related overdoses, there has been a dramatic increase of fentanyl-related, seized drugs being submitted and analyzed in crime laboratories. The structure of these drugs continues to be manipulated to generate new structurally similar substances challenging the rapid detection, identification, and reporting of these novel fentanyl substances, which is vital to the assessment and understanding of the illicit drug markets in specific locations.

Forensic scientists routinely utilize Electron Impact/Gas Chromatography/Mass-Spectrometry (EI/GC/MS) for the separation and identification of substances in drug samples; however, the use of GC/MS for the analysis of fentanyl samples comes with analytical challenges and limitations. Fentanyl analogs and their positional isomers have similar chemical structural configurations, making them difficult to detect and identify. GC/IRD is a useful and powerful tool in separating and identifying many of these compounds, for which traditional analytical techniques offer limited differentiations between them. In this study, it has been shown that similar fentanyl analogs (2- and 3- furanylfentanyl, 2-furanylbenzylfentanyl, crotonylfentanyl, cyclopropylfentanyl, methoxyacetylfentanyl, meta-fluoroisobutyryl fentanyl, and ortho-fluoroisobutyryl fentanyl) can be detected and identified using GC/IRD and optimization of GC/IRD parameters can enhance the resolution and detection of these substances. In particular, the effects of light pipe temperatures, resolution, and programmed temperature vaporizing have been studied.

#### **GC/IRD, Fentanyl, Analogs**



### B203 Data Mining the War on Drugs From Incarceration to Rehabilitation in Oklahoma

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**Learning Overview:** The goal of this presentation is to correlate changes in the law and incarceration trends over the past four decades in Oklahoma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by relaying the effect of new drug laws on the addicted population of Oklahoma.

The war-on-drugs era began in the 1980s and progressed through the 1990s and even through today. This era was remarkable due to courts giving harsher sentences for drug possession and distribution, which caused the prison population to grow from approximately 300,000 to 800,000 in only a decade.<sup>1</sup> The 2017 Bureau of Justice Statistics report showed there were more than 1,439,808 incarcerated individuals. The number of people incarcerated for drug offenses was 40,900 in 1980 and 452,964 in 2017.<sup>1</sup> This increase has caused overpopulation in the prisons and very little hope for rehabilitation. In 2017, the State of Oklahoma passed legislation reducing possession drug charges to misdemeanors in an effort to rehabilitate through drug treatment programs instead of prison time. The theory is that fewer individuals would be incarcerated on drug charges and that some may even receive help that could reduce recidivism and crime escalation. The fees for their misdemeanor charges were to be used to help with rehabilitation programs. The data mining study started with a sample size of 50 randomly selected individuals from Oklahoma that was selected from each of the decades of the 1980s, 1990s, 2000s, and 2010s. There was also an additional data group selected from 2017 to 2019, when possession of drugs was reduced to a misdemeanor. In addition to gender and ethnicity, data were broken down into the most common crime categories: Assault & Battery, Possession/Distribution of Controlled Drug Substances (CDS), Larceny, Burglary, Robbery, and Homicide/Manslaughter. Data were compiled and used to report the highest crimes from each category. There were also data on drug charges and the typical incarceration time seen over the decades. The uniform crime report provided by the Oklahoma State Bureau of Investigation showed a 7.5% decrease in drug arrests from 2016 to 2017.<sup>2</sup> Sample-sized data over the 1980–1990 periods suggested that Burglary and Possession of CDS had the highest frequency/count and may be linked, and this pattern continued over the decades. Newer data from 2017 did not show as significant a decrease in drug charges as expected, probably due to the lagging court system. However, data from 2018 is slowly showing a decrease in drug charges in comparison to previous years. As 2019 data are still being collected, it seems that drug charges may actually be slightly decreasing, especially when referring to possession charges. Tracking the changes over time may determine the effectiveness of the newly instituted misdemeanor drug laws in Oklahoma: 2018 total drug arrests—35,596; 2017 total drug arrests—38,996; 2016 total drug arrests—42,182; 2010 total drug arrests—37,398; 2006 total drug arrests—41,164; and 2002 total drug arrests—40,662.

#### Reference(s):

- <sup>1.</sup> *Key Statistics: Total Prison Population*. Bureau of Justice Statistics, accessed July 28, 2019, <https://www.bjs.gov/index.cfm?ty=kftp&tid=1>.
- <sup>2.</sup> *Crime Statistics*. Oklahoma State Bureau of Investigation, accessed July 28, 2019, <https://osbi.ok.gov/publications/crime-statistics>.

#### Oklahoma Drug Laws, Incarceration vs. Rehabilitation, War on Drugs



### **B204 An Advanced Extraction Method for Cyanide Metabolite Using Magnetic Carbon Nanotubes Facilitated Dispersive Micro Solid Phase Extraction (Mag-CNT/d- $\mu$ SPE)**

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**Learning Overview:** After attending this presentation, attendees will better understand an advanced extraction process for the extraction of a cyanide metabolite from biological samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) reviewing the potential use of the cyanide metabolite 2-Aminothiazoline-4-Carboxylic Acid (ATCA) as a forensic marker in cyanide exposure and in medicolegal death investigation; and (2) demonstrating advancement of Mag-CNT/d- $\mu$ SPE in extracting ATCA from biological samples using a one-step derivatization approach.

Cyanide intoxication can occur due to accidental exposures in industrial settings, consumption of cyanide-containing foods, or by illegal uses of cyanide in suicide, homicide, or terrorist acts. Sensitive analytical techniques have been developed to detect cyanide from biological samples. However, due to the unstable nature and incomputable kinetics of cyanide in biological samples upon storage, the analysis of its metabolites is considered to be more feasible for the purpose of exposure confirmation. The detection of the minor metabolite of cyanide, ATCA, has been proposed as an alternative method to determine cyanide exposure because of its stability and specificity. Studies have been extensively published to extract ATCA using conventional Solid Phase Extraction (SPE) and Liquid-Liquid Extraction (LLE). This research of ATCA extraction based on d- $\mu$ SPE has also been demonstrated to be promising for biological samples. In this work, a brief review of studies on the potential use of ATCA as a forensic marker will be discussed, and the performance of an advanced Mag-CNT/d- $\mu$ SPE in extracting ATCA from biological samples will be presented.

The Mag-CNT used in this study were synthesized in the Sam Houston State University research laboratory. The application of Mag-CNT/d- $\mu$ SPE to extract ATCA from biological samples was found promising and has been published. In this work, the combination of desorption and derivatization steps of ATCA from the Mag-CNT surface into a one-step process was investigated. Deionized water was used in method development and synthetic urine was used in optimization steps. In a typical extraction process, 5mg of Mag-CNT was used to extract ATCA from 500uL of biological sample in a microcentrifuge tube, which was spiked with known amount of ATCA and its isotopic internal standard, ATCA-<sup>13</sup>C, <sup>15</sup>N. The samples were then acidified with hydrochloric acid, vortexed, and sonicated for d- $\mu$ SPE for 10min. After d- $\mu$ SPE, the Mag-CNT were isolated with the aid of a strong magnet and were dried at 65°C under vacuum. N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was added to the dried Mag-CNT to derivatize and desorb ATCA, and the derivatized extracts were subjected to Gas Chromatography/Mass Spectroscopy (GC/MS) analysis. Optimization of the extraction parameters, including the amount of Mag-CNT, sample and derivatization volume, extraction and derivatization time, and dilution solvent systems was performed in triplicates.

The advanced Mag-CNT/d- $\mu$ SPE with the one-step derivatization approach was capable of reaching the detection limit of 5ng/mL and has a linear dynamic range between 10–1,000ng/mL, which is capable of detecting human endogenous level of ATCA in urine samples. The average extraction efficiency of 97.0% was obtained with a satisfactory bias and precision of  $\pm 20\%$  at the low, medium, and high level of quality control samples. It is envisioned that this advanced extraction platform will provide an additional tool for the quantitation of ATCA from biological samples with GC/MS analysis in forensic settings.

**Cyanide Metabolite, 2-Aminothiazoline-4-Carboxylic Acid, Magnetic Carbon Nanotubes**



### **B205 Direct Analysis in Real-Time Mass Spectrometry (DART®-MS) and Atmospheric Solids Analysis Probe/Mass Spectrometry (ASAP/MS) for the Detection and Analysis of Seed-Based Toxins**

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**Learning Overview:** After attending this presentation, attendees will have an understanding of how two ambient ionization MS systems could be used for the analysis of seed-based toxins in pure form or in the presence of the complex seed or seed mash matrices. This type of analysis could be crucial in suspected poisoning cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the necessary methods and capabilities for the detection of seed-based toxins in suspected poisoning cases through a rapid analysis that does not require any sample preparation.

Atmospheric Ionization Mass Spectrometry (AIMS) techniques (e.g., Direct Analysis in Real Time Mass Spectroscopy [DART®-MS] and Atmospheric Solid Analysis Probe/Mass Spectrometry [ASAP/MS]) have demonstrated utility for varying applications, such as psychoactive plant drug analysis and plant seed species identifications. These methods have been shown to be significantly faster than conventional methods without the need for sample pre-processing or derivatization steps. In cases in which poisonous plant evidence is to be identified, morphological characteristics are typically used in the initial presumptive identification. However, when there are no morphological characteristics, such as the case of seed mash, techniques such as DART®-MS or ASAP/MS may be helpful to presumptively identify possible poisons associated with the evidence. The chemical information from this type of presumptive analysis would aid toxicologists in deciding which extraction protocol (e.g., organic, acidic, or alkaline) to use for the isolation of the toxin from the mash. This would streamline the process of identification of the toxin present in the evidence.

This work provides the foundation to demonstrate the use of AIMS techniques (namely DART®-MS and ASAP/MS) for chemical analysis of seed-based toxins. In this project, a total of seven different toxins from five different seed species were targeted. Development of optimized methods for analysis required the ability to detect a large range of molecular weights and volatilities, necessitating some modifications from traditional analysis, specifically for DART®-MS. The use of techniques such as Infrared Thermal Desorption (IRTD) DART®-MS were employed to heat the sample to temperatures necessary for analyte desorption. From the optimal methods, instrument limits of detection of sub-nanogram to tens of nanograms was exhibited for DART®-MS, along with single to tens of micrograms per milliliter for ASAP/MS.

Analysis of actual seeds and seed mashes highlighted the rapid and sensitive analysis of these techniques for a presumptive test such as this. Detection of the toxin or toxins in the seeds was readily achievable, in most instances. For DART®-MS, samples were able to be analyzed as either an intact seed, dry seed mash, or extracted seed mash. For ASAP/MS, both the dry seed mash and extracted seed mash were capable of being analyzed, though for both techniques, the use of the extracted mash was superior.

The chemical information produced by these techniques also may allow for species identification. The mass spectral profile produced from all seeds analyzed, which included seeds from the same genus (i.e., *D. innoxia* and *D. stramonium*) and seeds of the same type (i.e., oleander) were compared using linear discriminant analysis. Differentiation of the species based on the chemical signatures was readily achievable in this instance, highlighting the ability to provide more information than just the toxin present. Current work is focusing on additional evaluations of this capability and on the use of random forest analysis to identify the most discriminative peaks for targeting purposes.

The use of AIMS techniques in the detection of seed mashes for the presumptive identification of toxins has not been demonstrated before. However, given the successful results of this work and the increasing usage of AIMS techniques, especially DART®-MS, in forensic laboratories, this offers a unique opportunity to deliver rapid presumptive information to toxicologists and investigators. The ability to also be able to obtain species-specific information using these tools could provide crucial information in these investigations.

#### **Toxins, Mass Spectrometry, Toxicology**





### **B206 The Determination of Total Tetrahydrocannabinol (THC) Concentration in Plant Material Via High Performance Liquid Chromatography With Ultraviolet Visible Diode Array Detection (HPLC-UV DAD)**

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**Learning Overview:** After attending this presentation, attendees will understand the process of quantitative method development and validation, the extraction of THC's from plant material for quantitation, and examples of practical application involving casework performed by the Colorado Bureau of Investigation (CBI) Forensic Science Laboratory.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a guide for laboratories seeking to develop and implement a THC quantitation procedure for plant material.

The State of Colorado, per the Colorado Revised Statutes (CRS) 35-61-101, defines industrial hemp as “a plant of the genus *cannabis* and any part of the plant, whether growing or not, containing a delta-9-tetrahydrocannabinol (THC) concentration of no more than three-tenths of one percent (0.3%) on a dry weight basis”. Colorado statute SB17-090 indicates that the delta-9-THC concentration is determined by measuring the combined concentration of delta-9-THC and delta-9-Tetrahydrocannabinolic Acid (THCA). Colorado’s legalization of medical and recreational marijuana, as well as a thriving hemp industry, has created a need for the development and utilization of scientific methods to distinguish hemp from marijuana. Primarily focused on the critical value of 0.3% THC as defined by the CRS, an HPLC-UV DAD method was developed and validated by the CBI to evaluate plant materials with total THC concentrations between 0.1% and 10%.

Samples are initially analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) prior to quantitation. The quantitative method involves the concurrent preparation of a six-point calibration curve, three positive aqueous controls, two plant process controls, and case samples (prepared in duplicate). Sample preparation consists of an initial drying step, grinding of the plant material, thermal decarboxylation of THCA to THC, and extraction in methanol. Androstenedione is used as an internal standard. Additionally, samples undergo a profiling step *prior* to decarboxylation by HPLC-UV DAD. This step allows the analyst to understand the components of the sample and, when compared with the quantitated samples, demonstrates that complete thermal decarboxylation of THCA was achieved.

Analysis of case samples utilizing this method has produced a surprising variety of results. The laboratory has analyzed samples that were quantitated below the lower quantitative limit of the method (0.1% total THC's) as well as samples above 10% THC. Perhaps most surprising has been the number of *true* hemp samples received by the CBI for analysis and the variation observed in terms of their overall cannabinoid profiles and physical appearance.

The precision of the method was determined using ten replicate injections of six THC solutions with concentrations ranging from 5 to 250 $\mu$ g/mL ( $n=60$ ). The Relative Standard Deviation (RSD) was calculated at each concentration and all were less than 3%. The accuracy was determined by comparing one set of six calibrators with the corresponding target concentrations ( $n=6$ ). Again, the RSD was calculated for each level and determined to be less than 3%. The measurement of uncertainty for the method is 8.31%, reported as a relative percentage of the total THC concentration, and was determined using replicate samplings ( $n=32$ ) of a known plant sample (0.32% THC) obtained from the Colorado Department of Agriculture. The CBI has created a robust method for the determination of total THC concentration in plant material.

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#### **Tetrahydrocannabinol, Quantitation, HPLC-UV DAD**



### **B207 High-Performance Thin-Layer Chromatography (HPTLC) Densitometric Analysis of Cannabinoids in *Cannabis sativa* L.**

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**Learning Overview:** The goal of this presentation is to report the results of the evaluation of different mobile phase systems for the analysis of cannabinoids in *Cannabis sativa* L. using HPTLC, as well as a method to differentiate marijuana samples from hemp samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reporting the results of the evaluation of ten mobile phase systems used in the HPTLC analysis of cannabis products. The most useful HPTLC systems for the analysis of cannabinoids in *Cannabis sativa* L. in forensic casework will be presented.

TLC is a planar chromatographic method that has been used as a routine, quick-and-easy tool for screening seized drugs in forensic casework. However, historically, traditional TLC has resulted in poor resolution due to systematic errors from hand-spotting, temperature/humidity control, and measurement of the Retardation factor ( $R_f$ ). HPTLC has been applied in the pharmaceutical field for years, yet it is not widely used in forensic science field. HPTLC has the potential to provide better resolution of forensic samples and to generate reports for more convenient documentation for peer review of casework.

There has been a variety of methods used to analyze cannabinoids by TLC, but the literature is void of a critical review of the different mobile phase systems. This presentation will report the results of the evaluation of ten different mobile phase systems for the analysis of cannabinoids in *Cannabis sativa* L. using HPTLC. All systems were run in triplicate.  $R_f$  were recorded and the resolution was calculated for 11 different cannabinoid certified standards. A CAMAG® HPTLC instrument setup was used for all analyses. The setup included an Automatic TLC Sampler 4, an Automatic Development Chamber 2, a TLC visualizer, and a TLC Scanner 3. HPTLC silica gel 60 F<sub>254</sub> 20 x 10cm plates were used for nine systems and an RP-18 WF 10 x 10cm plate was used for one additional system. Parameters for plate development included 30 seconds pre-drying, 20 minutes of humidity control and tank saturation with mobile phase, 70mm migration distance, and 5 minutes of drying after development. Samples were visualized on the plate under white light and at 254nm and 366nm wavelengths. A CAMAG® Chromatogram Immersion Device III was used to dip the developed plate into 0.5% Fast Blue B salt solution for five seconds. visionCATS CAMAG® HPTLC software (version 2.5) was used to control, document, and analyze all results from experiments. Two systems, xylene-hexane-diethylamine (25:10:1) and 6% diethylamine in toluene gave the best results in separating the three major cannabinoids,  $\Delta^9$ -*trans*-tetrahydrocannabinol ( $\Delta^9$ -THC), Cannabidiol (CBD), and Cannabinol (CBN). The results of the analysis of various cannabis products from casework will be presented.

The xylene:hexane:diethylamine (25:10:1) system was used to develop a calibration method for  $\Delta^9$ -THC. Regression analysis showed a linear relationship ( $R^2 > 0.99$ ) with a range of quantitation between 50–500ng. Marijuana samples and suspected hemp samples were able to be differentiated based on color intensity of sample when compared to standard calibrators of  $\Delta^9$ -THC and CBD.

HPTLC is a superior method to lower-resolution traditional TLC systems for qualitative identification of the common cannabinoids in cannabis products submitted to crime laboratories. It can also be used for quantitative analysis of THC in cannabis products when necessary. Using the proper mobile phase system with HPTLC can eliminate systematic errors from lower-resolution TLC, increase resolution, and make documentation easier. It is possible to differentiate hemp from marijuana, which could be very useful in cannabis casework, given recent legislative changes.

#### **HPTLC, Cannabinoids, Mobile Phase**



### B208 A New Analytical Scheme for the Analysis of Cannabis Plant Material: Marijuana or Hemp?

Sandra E. Rodriguez-Cruz, PhD\*, Drug Enforcement Administration, Dulles, VA 20166

**Learning Overview:** The goal of this presentation is to provide an overview of the new Drug Enforcement Administration's (DEA's) scheme for the analysis of cannabis submissions and the distinction between marijuana and hemp materials.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing a new analytical scheme for the analysis of cannabis.

Prior to December 2018, DEA laboratories used a three-tier analytical scheme for the analysis of cannabis submissions. This scheme consisted of macro and microscopical tests, the Duquenois-Levine (DL) color test, and separation analysis using either Thin-Layer Chromatography (TLC), Gas Chromatography/Flame Ionization Detector (GC/FID), or Gas Chromatography/Mass Spectrometry (GC/MS). For many years, this protocol for analysis provided the scientific foundation for reporting cannabis conclusions.

On December 20, 2018, the President of the United States signed into law the Agriculture Improvement Act of 2018 (Farm Bill), and with it, two significant changes were introduced. The new law includes an explanation of the term "hemp," now defined as "the plant *Cannabis sativa* L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9 tetrahydrocannabinol (THC) concentration of not more than 0.3 percent on a dry weight basis." As a second significant change, the new law also excluded the term "hemp" from the definition of marijuana in the Controlled Substances Act. These federal law changes directly affected the manner in which suspected cannabis submissions are analyzed by forensic chemists because the previously established analytical testing requirements—albeit sufficient for cannabis identification—were not fit for the purpose of assessing the level of THC present in a sample and, therefore, fell short of distinguishing between marijuana and hemp. In other words, unlike many other routine drugs encountered by analysts, the new "Farm Bill" requires the assessment of a quantitative property in order to provide a qualitative conclusion regarding the identification of marijuana submissions.

As a result of the federal law changes described above, a new analytical scheme was designed for the analysis of suspected cannabis materials submitted to DEA laboratories. In this presentation, this new testing protocol will be discussed. The new scheme includes macro and microscopical analysis, a new "typification" color test, and GC/MS analysis. The new color test allows differentiation between the two main chemotypes of cannabis by producing a pink color when the CBD concentration in the material is greater than THC (CBD > THC) or a blue color when THC > CBD. The GC/MS test is a limited-purpose method used to assess if the level of THC in a sample is above or below a decision limit of 1% (w/w). This presentation will include information on the background and validation of the new "typification" color test and on the scope, design, and validation of the GC/MS method. The discussion will also describe implementation of the new analytical scheme throughout DEA laboratories, as well as sampling and reporting requirements.

#### Cannabis, Marijuana, Hemp



## B209 Forty-Plus Ways Not to Analyze Beverages for Cannabinoids

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**Learning Overview:** After attending this presentation, attendees will understand the factors involved in selecting a method for the analysis of cannabinoids in beverages.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting approaches to determining acceptable methods for analyzing marijuana-infused beverages.

**Background/Introduction:** In the past decade, use of cannabinoids in the United States has increased tremendously. This increase has been primarily in marijuana-infused products, initially as food products and now as beverages. These products contain the psychoactive drugs delta-9-Tetrahydrocannabinol (THC) and/or Cannabidiol (CBD), and other cannabinoids. They are marketed and sold for their reputed medical and recreational properties. Regulation of these beverages exists only in the states in which they are legal. Federally, the United States Drug Enforcement Administration (DEA) has classified marijuana as a Schedule 1 substance. There is only one each United States Food and Drug Administration (FDA)-approved formulations of THC and CBD. In 2018, the Agriculture Improvement Act (Farm Bill) legalized hemp. This legislation does not address the formulation of marijuana-infused beverages, nor does it address the standardization of methods for potency analysis of these beverages. There is an increased need for accurate methods to determine THC and CBD content in these beverages. The three most common beverage matrices involve fermented (beer/ale), brewed (tea/coffee), and high sugar (soft drinks). Each of these matrices creates its own analytical challenges. Simple dilution methods with an aqueous or organic solvent are not plausible options for all beverages due to the potential for varying complex matrices (i.e., plant material, pulp, sugar, etc.), and the low solubility of cannabinoids in aqueous solvents.

**Objective:** To develop a method for the forensic analysis of cannabinoid-infused beverages.

**Methods:** More than 40 different methods were evaluated for Process Efficiency (%PE). These included simple dilutions, rapid solid Phase Extraction (SPE), and Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) methods, in which the aqueous and organic solvents were substituted or buffered in each method. To account for the low solubility of cannabinoids in aqueous solvents, the beverage was prepared by solubilizing the cannabinoids in an emulsion (surfactant [2% fruit pectin], carrier oil [12.6 % canola oil]), then adding the beverage (85.40%). %PE was determined at 14mcg/mL (5mg serving [ $n=3$ ]) in two different sets of samples for each extraction method. The before set was fortified before the extraction method, and the Non-Extracted External (NEET) standards were prepared in methanol. The %PE was determined using the post-extraction addition method. The %PE was calculated by using the average peak area of the before-fortified samples and dividing by the average peak area of the NEET samples, then multiplying by 100. An extraction method had an ideal %PE if the determined %PEs were within 75%–125% for all three matrices evaluated. A method had an acceptable %PE if determined %PE variations in all three matrices was  $<\pm 15\%$ . Samples analysis was performed using a previously presented and published high-performance liquid chromatography/tandem mass spectrometry cannabinoid method.

**Results:** In most extraction methods, %PE was similar between the fermented and brewed matrices, but the high sugar matrix was more than 20%–50% higher. The %PEs for the United Chemical Technologies Clean Screen FAST/THC 200mg/3mL (UCT THC) column for fermented, brewed, and sugar matrices were 40, 45, and 54%, respectively. Sample preparation involved 25mL of beverage, 225mL water, and 250mL acetonitrile, which was added to the UCT THC column and eluted with 80psi of air. The eluate was collected and analyzed.

**Conclusion:** No extraction method was determined to be ideal, but the UCT THC column was acceptable.

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### Cannabinoids, Beverage, THC



### **B210 An Analysis of Illicit Drugs by Portable Ion-Trap Gas Chromatography/Mass Spectrometry (GC/MS)**

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**Learning Overview:** After attending this presentation, attendees will understand the value of portable ion-trap GC/MS for the on-scene analysis of illicit drugs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science and law enforcement community by examining the benefits and limitations of field deployable ion-trap GC/MS for the analysis of illicit drugs.

False positive results from on-scene illicit-drug analysis using presumptive color tests have caused numerous wrongful arrests. In many states, forensic laboratories do not ever receive evidence for confirmatory identification of the illegal substance unless the defendant goes to trial, which makes it impossible to know the severity of this problem. Additionally, some defendants take a plea deal, which can result in these defendants spending months or years in prison for a crime they did not commit. Improving the reliability of on-scene illicit-drug testing by incorporating confirmatory methods capable of achieving very low limits of detection, GC/MS into the field could help reduce these wrongful arrests.

The purpose of this research was to build a GC/MS spectral library of illicit drugs and some common additives for a commercially available portable ion-trap GC/MS. The instrument uses a toroidal ion trap, which has three major advantages over other portable GC/MS systems: (1) its small size, which is essential when designing an instrument optimized for portability; (2) its durability due to having no components that require the highly precise alignment of the standard quadrupole system; and (3) its higher operating pressure, which lowers pumping requirements. Due to the potential for space charge and other ion-ion interactions in ion-trap MS, a customized library for this type of field application is critical. Space charge occurs when too many ions are trapped in a small volume causing problematic ion-ion repulsions that can result in a shift in observed  $m/z$  values. Other ion-ion interactions can occur inside the trap, such as when ion fragments complex with each other, resulting in observed  $m/z$  values that are greater than those expected from the initial fragmentation that occurs during the ionization process. Since space charge and other ion-ion interactions may potentially occur with ion-trap systems, an electronic GC/MS library created using data collected from an ion-trap GC/MS is a necessary supplement to the National Institute of Standards and Technology (NIST) MS database because the NIST MS database is primarily comprised of quadrupole mass spectra. If the potential of space charge and ion-ion interactions is not accounted for during the library-search process, this behavior may result in a missed identification (e.g., false negative).

In this research, more than 50 common illicit drugs and 15 additives were used to create an ion-trap GC/MS library after repeated testing. Many of the tested compounds had mass spectra different from that of the NIST MS database due to the presence of space charge and/or other ion-ion interactions. The ion-trap GC/MS library was then used to test the ability to detect and identify illicit substances and their additives in seized drug samples. In addition, results were compared with data generated from the same samples using a field-portable quadrupole GC/MS. It was concluded that with additional library development, portable ion-trap GC/MS is a viable choice for the confirmatory identification of seized drugs in the field.

#### **Illicit Drugs, Portable GC/MS, Ion-Trap GC/MS**



### **B211 New Psychoactive Substances in Forensic Drug Cases: Crossing the Borders of Gas Chromatography/Mass Spectrometry (GC/MS) Selectivity**

*Ruben F. Kranenburg, MS\**, Dutch National Police, Amsterdam, NH 1014BA, NETHERLANDS; *Arian C. Van Asten, PhD*, University of Amsterdam, Amsterdam 1098XH, NETHERLANDS

**Learning Overview:** After attending this presentation, attendees will understand the limitations of traditional techniques for drug identification in the current market comprised of many isomeric substances; gain insight into novel tools to add complementary selectivity to GC/MS, such as Gas Chromatography-Vacuum Ultraviolet (GC-VUV); learn about strategies of “crossing borders” of traditional GC/MS selectivity; and get more out of current available data using chemometric tools and Likelihood Ratio (LR) calculations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community, specifically drug experts, by enabling them to identify and overcome possible isomeric challenges in drug isomer analysis, reducing the risks for false positive or false negative results in case reports.

The global drugs-of-abuse market is facing major changes over the past decade. New Psychoactive Substances (NPS) form an emerging group of more than 700 synthetic drugs and are comprised of many closely related and isomeric classes of compounds. Most of these compounds are not legally controlled and are sold as “legal highs,” while others are banned substances in various countries. When a “legal high” is put under judicial control, other closely related yet uncontrolled compounds often increase in popularity and occurrence. This fuels a perpetual cycle in which increasingly diverse NPSs are continuously being developed.

This transformation of the drugs-of-abuse market changes the needs of forensic drug analysis laboratories. Established methods, such as GC/MS, fall short in terms of selectivity, as isomeric NPS have identical masses, very similar fragmentation spectra, and can co-elute in fast screening methods. Traditional spectroscopic measurements using Fourier Transform Infrared (FTIR) or Raman find limitations in multicomponent mixtures, as is often the case in adulterated samples or tablet formulations. Thus, in forensic laboratories, a need has arisen for analytical methods capable of distinguishing known, as well as identifying yet unknown, NPS isomers.

Three new strategies have been investigated in the Dutch National Police and University of Amsterdam groups to tackle the isomeric NPS dilemma: (1) VUV spectroscopy provides distinctive and very reproducible spectra for various ring-isomers. Selectivity-wise, GC-VUV is complementary to GC/MS for certain NPS classes consisting of both ring- and aliphatic chain isomers; (2) low-energy Electron Ionization (EI) on a Gas Chromatography/quadrupole Time-Of-Flight (GC/qTOF) system produces less fragmented, more information-rich, mass spectra for NPS isomers. Multivariate statistics were applied to discriminate among ring-isomers; and (3) Infrared Ion Spectroscopy (IRIS) at the FELIX laboratory successfully differentiated ring-isomeric forms of NPSs and correctly identified an NPS directly from a complex case sample. Finally, IRIS presents a promising approach for the identification of unknown NPSs for which reference standard compounds are not available when combined with quantum chemical prediction of IR spectra for candidate molecular structures.

#### **GC-VUV, NPS, Isomers**



### **B212 Implementing Raman Spectroscopy as a Tool to Characterize Sexual Assault Lubricants**

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**Learning Overview:** This project is an extension of the National Center for Forensic Science Sexual Assault (NCFS SAL) database. The database provides analytical data of sexual lubricants obtained from Direct Analysis in Real Time-Time-of-Flight/Mass Spectrometer (DART®-TOF/MS), Gas Chromatography/Mass Spectrometer (GC/MS), and Fourier Transform Infrared Spectroscopy (FTIR). After attending this presentation, attendees will understand the significance of Raman spectroscopy for screening sexual assault lubricants and its ability to classify unknown lubricants.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a supplemental method to identify trace lubricant residue based on its distinct “chemical fingerprints.”

More than 10 % of sexual assault cases reported involve condom use. In these instances, the chance of retrieving DNA evidence becomes minimal, and, therefore, other pertinent evidence, such as trace lubricant, can assist with justification. Previous research of the NCFS lab demonstrates the confidence of identifying and classifying trace lubricant based on its chemical constituents. Protocols were established using DART®-MS, GC/MS, and FTIR to distinctly associate various types of lubricants produced by multiple manufacturers. Further investigation elaborates on the ability to use chemical components as a bias to confidently classify lubricants into distinctive groups (e.g., flavored water-based, anesthetic water-based).

Raman spectroscopy provides molecular identification based on inelastic scattering from a monochromatic light (laser source). The change in frequency of a photon after interacting with the sample defines Raman scattering. Raman spectroscopy is non-destructive, highly sensitive, and reproducible with the appropriate parameters. These advantages appeal to forensic labs, which increases its use in identifying trace evidence, including textile fibers, paint, polymers, pigments and dyes, cosmetics, and explosives. Raman spectroscopy has presented itself as a useful tool in criminalistics, especially in the analysis of polymers, such as lubricants. Therefore, expanding the SAL database with spectral analysis of lubricants using Raman spectroscopy can prove beneficial.

Fifty samples inclusive of 21 Personal Hygiene Products (PHPs), 12 bottled lubricants, and 17 condoms were analyzed using Raman spectroscopy. The PHPs included body washes and soaps, whereas the lubricants were representative of two types: water-based and oil-based. Spectra were obtained on neat lubricants and extracts in triplicates via Raman spectroscopy with a laser beam at 785nm. Statistical analyses were performed using Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and Linear Discriminant Analysis (LDA) to classify the samples into groups based on shared components. After assessment of the neat samples, ten groups were established with 10% similarity based on the HCA dendrogram. Most of the samples clustered into groups could be identified by their obvious classes (lubricants vs. personal hygiene products); however, overlap was found, which will be discussed in the presentation.

#### **Raman Spectroscopy, Lubricants, Personal Hygiene Products**



### **B213 Assessing the Value of a Physical End Match in Trace Evidence: A Comparison of Human-Based and Computational-Based Approaches**

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**Learning Overview:** After attending this presentation, attendees will be informed regarding a developed systematic method for reporting the quality of a physical fit of duct tape edges, the results for inter- and intra-examiner variation in the reporting of a quantitative similarity score, and the comparison of human-based and computational-based approaches.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the value of physical fits in trace evidence. This presentation will also demonstrate the development of a systematic method on duct tape and highlight future research that aims to expand the materials and methodologies applied to the analysis of edges to offer additional support to inform the examiner's expert opinion.

Physical match examinations have played a critical role in forensic science for many years and extend across many forensic disciplines. The analysis of a potential physical fit involves an examination of separated edges to determine if the compared items re-align with distinctive features. In trace evidence, physical fits represent the highest degree of association between two items of evidence. As such, many examinations end once a fit is discovered and documented. Currently, physical fit examinations are being appraised to introduce a more standard and systematic methodology to support the opinions of examiners.

This work expands on the research presented by the West Virginia University group at the 2019 American Academy of Forensic Science conference regarding the comparison of duct tape edges using a systematic and quantitative method through an increased sample set, additional methods of interpreting the data, and assessment of intra-examiner and inter-examiner variation. In this study, edge similarity scores were utilized to assess 2,500 duct tape edges of varying grade, separation method, and degree of stretching. Edge similarity scores were calculated as a relative ratio of observed matching sections per scrim area. The performance rates of the method were calculated, and the distributions of scores were assessed to interpret the quality of a fit between edges. Overall, the method produced accuracies of between 84.9%–99.8% for the different tapes sets, with no false positives reported. It was found that the grade of the tape had a substantial impact on the quality of a fit between edges.

The distributions of edges were assessed utilizing Receiver Operator Curves (ROCs), boxplots, kernel density functions, beta distributions, and score likelihood ratios, demonstrating that a score of 80% or higher indicates strong support for a match, while scores below 25% indicate strong support for a non-match. Intra-examiner and inter-examiner variation were evaluated on blind sets of tape ends and agreement better than 15% was observed between reported edge similarity scores. Additionally, a machine learning algorithm was developed to predict if images of two pieces of duct tape coincide. The algorithm is based on Artificial Neural Networks (ANN) using the open-source python package scikit-learn. To train and use the ANN, first, the connectivity pattern of the network was defined. The architecture is defined by the number of initial input nodes, number of hidden layers with their corresponding neurons, and, finally, the number of output neurons. In the preliminary architecture of the network, five hidden layers with 200 nodes in each layer were chosen, using a constant learning rate; 200 matched the torn images, and 200 images were classified as not matched. The training step used 80% of this data and the rest was used to evaluate the accuracy of the model. Within these parameters, accuracy of ~85% was achieved, which was comparable to the examiner's performance.

#### **Physical End Matching, Trace Evidence, Duct Tape**



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**B214 A Survey of Elements Detected in Automotive Paint Layers by Scanning Electron Microscope/Energy Dispersive X-Ray Spectrometry (SEM/EDS)**

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**Learning Overview:** After attending this presentation, attendees will develop an understanding of the major, minor, and trace elements commonly detectable in automotive paint samples by EDS in an SEM.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community and, in particular, trace evidence analysts by providing an increased context by which to assess the significance of elemental data collected from automotive paint samples.

The elemental analysis of paint layers by SEM/EDS is a distinct step in the forensic comparison of automotive paint as defined by the American Society for Testing and Materials (ASTM) Guide E2809. While the elemental analysis of paint by SEM/EDS is utilized by many laboratories conducting paint analysis, case reviews and a review of several crime laboratory Standard Operating Procedures (SOPs) show that various stages of this process, from sample preparation through interpretation, could be better defined. A set of 300 automotive paint samples (i.e., street samples), comprising 1,203 individual layers, was employed to investigate each step of this process. Here, data on the elements that were detected in each layer of this automotive paint sample set are presented. While this is nominally a trivial process for major elements, the impact of closely spaced layers, the choice of analytical variables, and the challenges of objectively processing spectra demonstrate that this is not a trivial process for the determination of minor and trace elements.

Layers in each paint sample were delineated and identified as a clear coat, tinted clear coat, base coat, or primer by a combination of light microscopy and backscatter electron imaging. Using a thick section, each of the 1,203 layers (356 clear coats, 79 tinted clear coats, 347 color coats, and 421 primer layers) was then analyzed by EDS twice, first using livetimes of 20s and 200s. In both cases, analyses were performed from a manually drawn raster area within each layer. The resulting spectra were evaluated to determine which elements were detected in each layer. To avoid the subjectivity of manual peak assignments, this work evaluated several objective criteria for peak detection. Various combinations of the following approaches were compared: (1) a fixed element list, (2) an automated peak identification algorithm, (3) a 3-sigma detection limit threshold (based on the EDS counting error), and (4) fixed weight percent detection limit thresholds. Through iterative processing of the data, a fixed list of 24 elements was developed, which included every element detected in at least one of the 1,200 layers. Spectra from both the 20s and 200s livetime runs were then processed using three detection limits: 3-sigma (representing the lowest detection limit), 0.25wt-% fixed, and 0.50wt-% fixed (representing the highest detection limit). Using the highest detection limit (0.5wt-%) and a 20s analysis time, four elements (carbon [C], nitrogen [N], oxygen [O], and silicon [Si]) were detected in at least 40% of the clearcoats. Seventeen elements were detected in at least 1% of the color coats (C, N, O, magnesium [Mg], aluminum [Al], Si, phosphorus [P], sulphur [S], chlorine [Cl], potassium [K], calcium [Ca], titanium [Ti], chromium [Cr], iron [Fe], copper [Cu], zinc [Zn], and barium [Ba]). Seventeen elements were detected in at least 1% of the primer layers (C, N, O, Mg, Al, Si, P, S, Cl, Ca, Ti, Fe, Zn, tin [Sn], Ba, bismuth [Bi], and strontium [Sr]). The average number of elements detected in a given layer increased from the clear coat (four) to the color coat (seven) to the primer (nine). The 200s analyses increased the number of elements detected; however, this impacted only elements detected at low concentrations (i.e., near the detection limit).

These results have numerous practical applications: (1) each of the detected elements can be associated with an expected component in the paint (e.g., a binder, filler, pigment, or additive); (2) this data provides information about the frequency of particular elements in each layer of paint, as well as their anticipated concentration range in the paint; and (3) this dataset provides new insight into the significance of the suite of elements detected in a questioned automotive paint layer and sample, which may ultimately improve the value of elemental data obtained from paint samples.

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**Paint, SEM/EDS, Elements**



### B215 Parameter Optimization and Validation for Qualitative Elemental Analysis of Electrical Tape Backings by X-Ray Fluorescence (XRF)

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**Learning Overview:** After attending this presentation, attendees will have learned of the benefit of implementing XRF in the analysis of electrical tape backings due to the rapid and non-destructive nature of the method. Attendees will gain an understanding of the key factors and potential interferences to assess when attempting to adapt this method for use in their respective laboratories while learning of the relevant figures of merit reported for the method on an XRF instrument.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the validation process of a rapid and non-destructive method for increased sample discrimination of electrical tape backings beyond traditional Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) analysis.

Electrical tape becomes relevant forensic evidence in high-profile casework involving homicides or explosive devices. As such, it is critical that practitioners have access to rapid, minimally invasive techniques of analysis to best support these investigations. The characterization of electrical tape backings through XRF spectroscopy has been shown to be a highly discriminatory, non-destructive method of analysis requiring limited sample preparation. In a previous study by Prusinowski et al., XRF analysis of electrical tape backings exhibited increased discrimination from traditional SEM/EDS methodology from 78.8% up to 91.0%, making the method desirable for implementation in a forensic laboratory.<sup>1</sup>

This study describes the process of parameter optimization and validation of the method on an XRF spectrometer for casework use. This work expands upon the previous discrimination study by broadening the total sample set of characterized tapes from 40 to 90 samples. Replicate measurements on different locations of the tape sample were analyzed ( $n=3$ ) to assess the within-sample variability. XRF provided superior discrimination to SEM/EDS on the expanded dataset and a more comprehensive elemental characterization (17 elements by XRF vs. 8 by SEM/EDS). Duplicate controls and tape fragments from the same roll were also analyzed to assess inter-day, intra-day, and intra-roll variability. Parameter optimization included comparison of atmospheric conditions, collection times, and instrumental filters. A study of the effects of adhesive and backing thickness on spectrum collection revealed key implications to the method that required modification to the sample substrate. As an electrical tape standard reference material does not currently exist, the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1831, a standard soda-lime glass, was found to be an adequate reference material for tuning the instrument.

In addition to the parameter optimization, figures of merit assessed during the validation portion of this study included accuracy, precision, sensitivity, selectivity, and processed sample stability. By optimizing this technique for use on an XRF instrument, a new tool for rapid, highly informative elemental analysis of electrical tape backings was provided to expand examiners' casework capabilities.

#### Reference(s):

- <sup>1</sup>. Meghan Prusinowski et al. Assessment of the utility of X-ray Fluorescence for the chemical characterization and comparison of black electrical tape backings. *Forensic Chemistry* 13 (February 2019): 1-11, <https://doi.org/10.1016/j.forc.2019.100146>.

#### X-Ray Fluorescence, Elemental Analysis, Electrical Tape



### B216 A Quantitative Trace Elemental Analysis of Aluminum Materials for Forensic Discrimination

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**Learning Overview:** After attending this presentation, attendees will understand how trace elemental analysis of aluminum (Al) materials by Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) can be used for source discrimination of Al samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a method to measure the trace elemental composition of Al samples, which can be used to discriminate among various sources of aluminum.

**Hypothesis Statement:** Quantitative measurement of trace elemental concentrations in Al materials by ICP/MS can discriminate between sources and provide valuable information regarding the exclusion or potential association of two compared samples.

Al is commonly used as a metallic fuel in the chemical mixtures of Improvised Explosive Devices (IEDs) to increase the heat of explosion. Various types of Al-rich products, including foil, paste, powder, cans, spray paint, pyrotechnics, and binary exploding targets are easily accessible to the general public and can provide the starting material for production of IEDs. Al powder from these sources can be produced inexpensively using simple, available techniques. This research developed a method to accurately and precisely measure the trace elemental concentrations of Al materials and aims to evaluate its discrimination potential for various sources of Al through the comparison of their trace elemental profiles.

Koons et al. measured trace element compositions for 30 rolls of Al foil and determined that there were quantifiable differences among rolls from 12 discrete manufacturers.<sup>1</sup> In addition to being able to differentiate between manufacturers, different lots from the same manufacturer also displayed distinct compositions. This study builds on these initial results and develops a more robust determination of the discriminatory power of comparisons of Al trace element compositions. This will be achieved through increased sampling, sample diversity, and more sensitive instrumentation. The total number of samples included in the present study is larger (~350 sources, currently) and sample variability is greater in terms of source material and region. The instrumentation allows for greater precision and lower detection limits, potentially yielding additional elements for comparison.

Concentrations of trace elements are determined from solutions using High-Resolution-Inductively-Coupled Plasma-Mass Spectrometry (HR-ICP-MS). For sample types that necessitate separation, the Al component is isolated from the other components of the product. Approximately 50mg of purified Al is dissolved using high-purity hydrochloric and nitric acids. Three aliquots of these concentrated sample digestions are removed and diluted to ~500ppm of dissolved Al for analysis on a HR-ICP-MS. Measurements were obtained using external calibration and internal standardization. The calibration standards reflect a wide range of elemental concentrations that may be observed in Al samples. A multielement internal standard is used in order to correct for instrumental drift, signal suppression, and matrix effects resulting from the high Al matrix of the samples. National Institute of Standards and Technology (NIST) and International Analytical Reference Materials (IARM) Al alloy reference materials are used as external standards and are regularly measured to verify the accuracy of the method. Quantitative data for all analyte elements certified in the external reference materials are collected; non-certified analytes are also measured, though the accuracy of these measurements cannot be verified.

An initial test was conducted to determine the homogeneity within a roll of Al foil for the measured element concentrations. This test provides a means to assess the degree of sampling necessary for Al foil rolls measured in this study. Three samples were taken across the width of a roll at approximately every ten feet for the entire length of the roll (250ft), totaling 78 samples from a single roll of Al foil. These samples were digested and analyzed according to the established procedure.

Statistical analysis will be used in order to determine which elements will have the greatest effect on the discriminating power (which is equal to one minus the random match probability) when attempting to distinguish between Al sources. Within- and between-source distributions of trace elemental concentrations will be used to develop criteria to compare Al samples from two sources: one from the Al material recovered from a crime scene and the other from a specified known Al source. Further, a method to assess the evidential support for an association between materials recovered from a crime scene and a specified Al source versus an alternative source will be explored.

#### Reference(s):

- <sup>1</sup> Koons, R.D., Peters, C.A., and Merrill, R.A. Forensic Comparison of Household Aluminum Foils Using Elemental Composition by Inductively Coupled Plasma-Atomic Emission Spectrometry. *Journal of Forensic Sciences*, 38, no. 2 (1993): 302-315. doi: 10.1520/JFS13409J.

#### Trace Elements, Aluminum, ICP/MS



### B217 Vibrational Spectroscopic Analysis of 3D-Printed Polymers Pre- and Post- Manufacturing

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**Learning Overview:** After attending this presentation, attendees will understand the value of vibrational spectroscopy for the analysis of polymers used in the manufacturing of 3D-printed objects.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about the chemistry of polymers used in 3D printing, pre- and post-manufacturing, and how that information can be obtained through vibrational spectroscopy, thus informing criminalists about the significance of vibrational spectra of 3D-printed objects.

Additive manufacturing, commonly known as 3D printing, is becoming increasingly common in today's society. The ease of use and decreasing cost make this technology accessible to a wide range of individuals. 3D printing has been used to manufacture a range of legal items, including airplane and automobile parts, footwear, medical prosthetics, and even models for forensic reconstructions and court presentations. Unfortunately, it is also readily available for individuals to manufacture items that can be used in criminal activity, such as firearm components, knuckle dusters, pipe bomb components and ATM skimmers. In an investigation in which 3D-printed parts are recovered and a 3D printer is found, it would be valuable to be able to associate the printed object with an unused spool of polymer. Thus, there is a need for forensic-focused research to evaluate whether an association can be made between 3D-printed objects and their raw material (i.e., the polymer filament) as well as the discrimination potential of polymers produced from different batches and different manufacturers. It is the focus of this research to be able to understand the chemical nature of the polymers used in 3D printing, including changes in their crystallinity via the manufacturing process, in addition to methods for their classification and discrimination.

Polymer crystallinity refers to the degree of structural order or alignment of the molecular chains in a solid composed of repeating structural units. Different polymers have various ranges of degrees of crystallinity, which refers to the percentage of order within the solid. Polymers can crystallize upon cooling from the melt; thus, it is possible for there to be a change in polymer crystallinity as a result of the 3D manufacturing process. In order to associate a 3D-printed object with its polymer filament, it is imperative to understand any changes to the crystallinity of a polymer as a result of the 3D-printing process.

Infrared (IR) and Raman spectroscopy are common instruments used in forensic laboratories and are well known and validated analytical methods for polymer characterization and analysis. In addition to identifying the components of the polymer, such as the main chemical, additives, and pigments, vibrational spectroscopy has been used for analyzing polymer crystallinity. Changes in polymer crystallinity can result in peak splitting and changes in peak shape, which may result in a shift in the computer-selected peak position.

This research focused on the vibrational spectroscopic analysis of two common polymers used in 3D printing: Polylactic Acid (PLA) and Acrylonitrile Butadiene Styrene (ABS). For each polymer type, filament spools of different colors within the same brand and also different brands were analyzed. Pre-manufactured samples were analyzed from each spool at increments of 24in for a total of five pre-manufactured measurements per spool. Additionally, for all polymer filament spools, three items were manufactured at three different temperatures for a total of nine 3D-printed objects per spool.

IR and Raman spectral analysis resulted in the identification of the major and minor components (e.g., additives and pigment) of the polymers for classification as well as lot and brand comparisons. It was concluded that different brands and colors of polymers were able to be discriminated based on their vibrational spectra. Within a spool of polymer, there were no meaningful detectable differences, indicating that the polymers within a spool are homogeneous. Last, statistical comparisons of the peak positions, splitting and Full Width Half Max (FWHM) values of the pre- and post-manufactured polymers indicated that there were no consistent differences over the range of melting temperatures to indicate a measurable change in crystallinity via the 3D-printing process. Overall, vibrational spectroscopy was shown to be a valuable tool for 3D-printed polymer identification, which can be used to associate a 3D-printed object with a specific manufacturer's polymer filament.

#### 3D-Printed Polymers, Infrared Spectroscopy, Raman Spectroscopy



### **B218 Non-Destructive Separation of Pollen Grain Constituents for Biochemical Analysis**

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**Learning Overview:** After attending this presentation, attendees will understand a comprehensive separation protocol that furthers the use of pollen grain beyond microscopy (i.e., DNA and chemical analysis).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing additional avenues for the analysis of pollen grains in forensic laboratories at the state or local levels.

Forensic palynology, the study of pollen grain morphology, has been used in a variety of cases around the globe. Pollen grain evidence provides the ability to determine the location where people or objects have been by the unique assemblage that gets transferred onto them. This deposition can occur from direct contact with a plant or dirt samples and/or by airborne pollen. Current analysis in the field of forensic palynology relies on the use of visual microscopic analysis (optical microscopy or scanning electron microscopy) to determine the plant species a single grain originates from via its morphological features. Pollen grains are a multi-component structure consisting of an external pollen coat over a decay-resistant shell, containing its genetic material. To visualize the unique morphology of pollen grains, the coating and other external material must be removed. The most common technique is acetolysis. It uses a mixture of acetic anhydride and sulfuric acid to dissolve any organic and/or inorganic material from the grain surface. It also dyes the grain a dark brown hue for visual microscopic analysis. The removal of organic material from the pollen also destroys the pollen coat and DNA (via infiltration through the pollen apertures), leaving only the shell (more explicitly, exine and intine) of the pollen grain for microscopic analysis. This procedure prevents any additional analysis.

In recent studies, it has been known that analytical methods such as Inductively Coupled Plasma/Mass Spectrometry (ICP/MS), Raman, and Infrared (IR) absorption, as well as DNA analysis, can provide additional information for the identification of pollen species. But as mentioned above, current cleaning methods prevent any additional analysis due to the chemical destruction of the coating and the genetic material. Reciprocally, any DNA analysis has been done by crushing the grains, preventing microscopic and chemical analysis.

This presentation will show a simple cleaning method that maintains the pollen grain morphology while also providing material for additional spectroscopic and DNA information. Soxhlet extraction using ethanol as a solvent will be shown to be a method that allows for DNA and coating to be removed without damaging or altering the chemical integrity of both components. This presentation will show the complementary analyses obtained from every constituent of pollen grains from nine different species. Molecular information (from Raman and IR absorption), elemental analysis (from ICP/MS), DNA analysis (via quantitative Polymerase Chain Reaction [qPCR]) and microscopic analysis (via Scanning Electron Microscopy [SEM] and digital microscopy) can be combined for the classification of pollen species.

Such a simple and versatile preparation of pollen grains will indubitably bring the use of pollen as a forensic evidence back to the light and provide forensic examiners with new options for geolocation and association.

#### **Pollen, Palynology, Forensic Botany**

### **B219 Thanatochemistry at the Crime Scene: A Microfluidic Paper-Based Device for Ammonium Analysis in the Vitreous Humor**

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**Learning Overview:** After attending this presentation, attendees will be informed about the advantages of the use of paper-based microfluidic technology for inferring the Postmortem Interval (PMI).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new tool for estimating the time since death. Thanks to the low cost and portability of the developed devices, this method has the potential of offering a real breakthrough at the crime scene, offering, for the first time, the possibility of a rapid and objective estimation of the time since death after the equilibration of the body temperature with the environment (approximately 18–20 hours).

The estimation of the time since death is a crucial question that forensic experts need to answer directly at the crime scene to effectively address the investigations.

Notwithstanding a large number of studies regarding the PMI estimation, this problem is still faced using body examination. To obtain more accurate and objective data, after the first 20 hours during which body cooling is typically used, most of the literature is based on the analysis of the postmortem changes occurring in the vitreous humor, such as potassium estimation. Recently, the correlation between the concentration of the ammonium ion in the vitreous humor and the time since death was demonstrated.<sup>1</sup> Although the analytical methods offer sensitivity and specificity, at the moment, thanatochemistry analyses require the application of expensive and complex separative instruments.

Since the first introduction by Whitesides et al., the use of Paper-Based Microfluidic Devices ( $\mu$ PADs) for the development of chemical sensors has been extensively reported.<sup>2,3</sup> Among several approaches for producing  $\mu$ PADs, the use of commercial wax printers proved to be inexpensive and straightforward in the fabrication process. The procedure is based on two steps: (1) patterning chromatography paper into hydrophilic channels by fabricating hydrophobic barriers, and (2) the addition of the reagent to the hydrophilic portion of the paper support. The sample is driven through the reagent zone as results of the wicking capacity of the paper without external assistance. This paper-based technology proved to be rapid and easy-to-use. This approach does not require highly qualified personnel nor expensive instrumentation. Also, it can be performed onsite, also enabling a prompt analytical response in less-equipped environments. The advantages of  $\mu$ PADs have provided forensic science with reliable tools to face different forensic issues.

On the grounds of the above considerations, the goal of this presentation is to show the use of a low-cost device able to provide a rapid and sensitive colorimetric detection of the ammonium in vitreous humor. The developed procedure is conceived to obtain a first-line response for inferring the PMI before samples are sent to an equipped laboratory for further analysis. The preliminary results could be used to prioritize investigative directions.

The proposed microfluidic devices were designed as a single spot pattern. The test was based on a specific reaction for the detection of ammonium, which uses the Nessler's reagent. The vitreous humor sample is transferred without any treatment directly onto the hydrophilic portion of the paper, and a colorimetric reaction is developed in a few minutes. The color change is measured in terms of "Red, Green, Blue [RGB] distance" by using a simple and free application for smart phone cameras. The device was optimized to accurately and precisely quantify the ammonium concentration. The selectivity of the Nessler's reaction was tested toward the main vitreous humor components, and the stability over ten days was tested. The optimized device was used for the determination of ammonium ion in vitreous humor samples from forensic autopsies ( $n=25$ ). The results obtained with the proposed method were compared with the data obtained with an instrumental analysis based on capillary electrophoresis.

In conclusion, the results of the use of a paper-based microfluidic approach and the advantages of the device as an additional tool to the traditional methods for an objective, although still presumptive, estimation of the time of death directly at the crime scene will be shown during the presentation.

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2. A.W. Martinez, S.T. Phillips, M.J. Butte, G.M. Whitesides. Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angew. Chem. Int. Ed. Engl.* 46 (2007) 1318–20.
3. D.M. Cate, J.A. Adkins, J. Mettakoonpitak, C.S. Henry. Recent developments in paper-based microfluidic devices. *Anal. Chem.* 87 (2015) 19–41.

#### **Microfluidic Paper-Based Devices, Postmortem Interval (PMI), Thanatochemistry at the Crime Scene**



### C1 The Development of a Cleaning Protocol for Mobile Devices Damaged by Fire

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**Learning Overview:** After attending this presentation, attendees will understand the cleaning protocols developed to aid in handling mobile devices following fire damage and extinguishment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a best practice for evidence collection to safely handle, clean, store, and acquire data from mobile devices following fire damage and extinguishment.

Mobile devices damaged by fire can be problematic for forensic investigators. If the inner components of the mobile device are damaged or melted, the probability of collecting a successful cable acquisition decreases. In many cases, the outer shell and components of the device are easily destroyed, while the inner components of the mobile devices remain mostly intact, but blackened and soiled. In these cases, mobile devices may need to be cleaned utilizing a forensically sound protocol before they are sent to a forensic analyst.

Many fire marshals and fire departments do not have cleaning protocols for mobile and/or digital devices. Once collected and submitted to the forensic laboratory, digital devices may sit untouched for months due to backlog. Residue from extinguishers can be corrosive to devices and can damage the inner components. The goal for this project was to determine an appropriate, low-cost cleaning methodology and protocol for mobile devices damaged by fire that evidence collection teams, fire departments, and fire marshals can implement before forwarding the devices to the forensic laboratory for analysis.

The three most common ignition modalities are commercial gasoline, commercial diesel, and combustibles. Fire departments typically utilize water as the primary extinguishment method, while class A foam and dry chemical fire extinguishers are often the extinguishers used in a workplace setting. The ignition and extinguishment outlined were used during experimentation.

A total of 60 Blackberry® 8330 Curve and 9330 Curve 3 mobile devices were used during this experiment. All devices were initially imaged via traditional cable acquisition with Cellebrite® Universal Forensic Extraction Device (UFED) 4PC® version 7.18.0.199 and total file sizes were collected for the acquired physical image. The devices were then introduced to each of the methods of ignition, burned in a controlled setting, then extinguished. The devices were collected and cleaned utilizing various predetermined cleaning protocols. A Gas Chromatography/Mass Spectrometer (GC/MS) equipped with a Restek Rtx®-5MS column (30.0m x 250µm x 0.25µm) was utilized before and after cleaning each phone to confirm that there was no remaining residue from ignitable liquids or the extinguishers. Previous research determined a 15-minute cleaning cycle with 5% Elma Ultrasonics® Elma Tec® Clean A1 Solution followed by a 24-hour drying cycle is the ideal cleaning procedure for mobile devices that have been submerged in ignitable liquids.<sup>1</sup> Scrubbing Bubbles® was used as the second cleaning method for departments that do not have access to an ultrasonic cleaner. The third cleaning method used was a 95% solution of isopropyl alcohol because it is currently used in laboratories to clean water from the boards of mobile devices.

To determine that the data on the mobile devices remained intact after cleaning, the devices were re-imaged using cable acquisition as previously outlined or advanced acquisition methods (chip-off or JTAG) where cable acquisitions failed. For devices where acquisition was not possible, the conditions of the logic board and internal components were examined to determine whether it was fire/heat damage or the extinguishing chemical that prevented a successful acquisition. Using Cellebrite® UFED Physical Analyzer® version 7.7.0.93, the file sizes collected after cleaning were compared to the file size collected prior to damage to show that the data on the mobile device had not been changed.

#### Reference(s):

- <sup>1</sup> Nico Kresl, Chelsey McPhillen, HollyAnn Swann, et al. The Development of Cleaning Protocols for the Analysis of Mobile Devices That Have Been Submerged in Oil-Based, Flammable, and Clandestine Chemical Liquids. *Proceedings of the American Academy of Forensic Sciences*, 71<sup>st</sup> Annual Scientific Meeting, Baltimore, MD, 2019.

#### Mobile Forensics, Damaged Devices, Fire



### C2 Ear Asymmetry—A Preliminary Evaluation of Identification Accuracy for Forensic Purposes

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**Learning Overview:** After attending this presentation, attendees will have important updates about the correlation between measures of the ear and will have the opportunity to appreciate how these can be useful for personal identification in the forensic field.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by considering the possibility of a correlation between the measures relating to the ear and its application in the context of personal identification in the forensic field.

In the modern age, the identification of the living has become a major challenge in the field of forensic science. To achieve this goal, forensic sciences have focused on biometrics, a method to classify and identify the physiological and behavioral traits of an individual. The study of biometric parameters was focused for forensic purposes to ensure an accurate assessment in the identification process.

Facial features, such as the eyebrows, nose, lips, ears, or details, such as scars or tattoos, are useful parameters to identify a person of interest in the forensic context. These features are considered important because of their rarity in form, position, and size across different individuals.

Among these parameters, the ear has attracted much interest given its inter- and intra-individual uniqueness. The main challenge is regulated by defining how the uniqueness of this biometric parameter is distinctive for each individual. Forensic sciences have been studying this anatomical element for many years thanks to the non-intrusiveness of the methodologies provided. The analysis of the external ear makes it possible to observe and record its characteristics by simple observation and scanning. The uniqueness and permanence of the ear characteristics have been made into a biometric identifier. It has been observed that the shape and structure of the ear remain permanent from about 8 to 70 years of age.

The morphological and metric study of this body component demonstrates a high degree of individual uniqueness. However, the ear is often partially covered and thus difficult to visualize and measure. Furthermore, the resolution of surveillance video may not allow accurate measurement of some key components. This limits the use of the ear in some cases of video surveillance identification.

The present study aims to quantify intra- and inter-individual variability in metrics associated with the ear. By subjecting the ear to nine measurements, the goal is to study the extent to which these metrics differ between individuals and to quantify variation in the same individual between the right and left ears.

Nine ear measurements from 35 individuals with ages ranging from 4 to 76 years (mean=32.9, SD=15.9) were studied. For each measurement, the difference between left and right ears, the Coefficient of Variation (CV) across all individuals, and the correlation between each measurement were calculated. The association between each measurement and sex, age, height, and history of boxing or rugby was also examined.

Pearsonian correlation among the nine measurements ranged from 0.48 to 0.91, indicating the nine metrics are highly correlated within each individual. CVs ranging from 12.1% to 31.5% were found, suggesting some measures are more suitable for identification because of greater inter-individual variation. These data will help to develop these measures for the purposes of identification and differentiation among individuals.

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#### Ear Asymmetry, Personal Identification, Forensic Anthropology





### C3 An Investigation Into the Encoding and Encryption of Black Box Data on a DJI Spark

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**Learning Overview:** After this presentation, attendees will have a better understanding of the black box files obtained from a drone and the formatting of their encoding or encryption.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by attempting to identify patterns of obfuscation and encryption within black box files of a DJI Spark. In addition, several open source tools will be used to acquire, parse, and compare data to ascertain which tool or combination of tools provides the most amount of usable data.

With the increased use of Unmanned Aerial Vehicles (UAV), new methods of forensic data collection and analysis of UAVs and their associated devices have been developed. In an attempt to combat the unwanted access to sensitive device data by outside third parties, such as law enforcement, companies who make these UAVs have increased their security. This increased security requires new methods and techniques to be employed in order for information to be gleaned.

DJI, a Chinese technology company, is one of the most prominent suppliers of drones to the world. The drones sold by DJI range from professional drones aimed at experienced flyers to smaller user-friendly amateur drones. The specific type of drone used during this study was the DJI Spark, a small drone primarily made for taking short flights while capturing pictures and videos. This drone has the capability of being controlled by a remote control, cellular device, or motion control. For the purposes of this study, the Spark was connected to a Samsung™ SM-G9001® mobile device.

The cellular device was imaged using Magnet® ACQUIRE™ version 2.13.0.15121. Files from this device and the drone's on-board microSD card were imaged using AccessData® FTK Imager® version 4.2.0.13. The DJI Spark flight logs were obtained using DJI Assistant 2® version 1.2.5. This free DJI app provided files from the internal memory of the drone. Flight logs were exported in a single .DAT file while black box data was exported in series of .log files. The DJI .DAT file was then extracted using DatCon's ExtractDJI® version 1.4.2, which provided a series of FLYxxx.DAT files. These individualized files provided a three-digit number, in place of "xxx," that corresponded to a recorded flight on the drone.

The .DAT files were parsed through using various open source tools, including DatCon®, Airdata™, and DROP.<sup>1</sup> Using these programs, readable .txt, .log, and .csv files were created where information about a drone's systems and flight parameters were displayed. The amount of information displayed across the applications varied. Common information among the tools used included the time of flight, battery percentage in relation to the time, and the mode of flight the drone was using.

Files pertaining to the black box consisted of mostly text documents that displayed information in WidChar when opened in Notepad® version 6.1. The hex values of these files were observed using FTK Imager® and HxD Hex Editor® version 2.3.0.0.

Log files obtained from the companion cellular device contained information about the flights extracted from the drone. In addition to the flights pulled from the drone directly, the companion cellular device included information about previous flights that were inaccessible from the drone. Located in a folder for the DJI Go 4® application, a flight error propagation log was found containing error notifications and time stamps.

The results of this research will provide a better understanding of the obfuscation and encryption techniques employed by DJI. In addition, this study aims to obtain a greater insight of which open source tools may retrieve usable forensic data from a DJI Spark and its associated products.

#### Reference(s):

- <sup>1</sup> Devon R. Clark, Christopher Meffert, Ibrahim Baggili, Frank Breitingner. DROP (Drone Open source Parser) your drone: Forensic analysis of the DJI Phantom III. *Digital Investigation* 22, (August 2017), S3-S14, <https://doi.org/10.1016/j.diin.2017.06.013>.

#### Black Box, Drone, Encoded



## Digital & Multimedia Sciences—2020

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### C4 The Organization of Scientific Area Committees (OSAC) Digital/Multimedia Scientific Area Committee Standards Work—Part 1: Digital Evidence and Video/Imaging Technology and Analysis (VITAL)

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**Learning Overview:** After attending this presentation, attendees will have learned about the latest work in developing OSAC Standards for the Digital and Multimedia Sciences disciplines of Digital Evidence and VITAL.

**Impact on the Forensic Science Community:** Standards are vital to ensuring the sound practice of forensic science across all disciplines. In order to implement these standards, community members must know of their existence and scope. This presentation will impact the forensic science community by offering newcomers an introduction to such standards, while offering more experienced practitioners with an opportunity to probe the finer details of the current work and shape the direction of future work.

The OSAC for Forensic Science works to strengthen the nation's use of forensic science by facilitating the development of technically sound forensic science standards and by promoting the adoption of those standards by the forensic science community. These standards are written documents that define minimum requirements, best practices, standard protocols, and other guidance to help ensure that the results of forensic analysis are reliable and reproducible.

The OSAC forensic science disciplines are spread across five major "Scientific Area Committees" or "SACs." The SAC most relevant to the American Academy of Forensic Sciences (AAFS) Digital & Multimedia Sciences Section is the "Digital/Multimedia SAC" (DMSAC). The DMSAC incorporates four subcommittees that address the following disciplines: Digital Evidence, Video/Imaging Technology & Analysis, Facial Identification, and Speaker Recognition. This presentation will focus on the work of the first two, while a companion presentation addresses the second two.

Recently, the Forensic Science Standards Board (FSSB), which oversees the work of the various SACs and Subcommittees, directed each subcommittee to establish a "roadmap" of standards for their discipline. Each roadmap would identify the individual standards considered to be of the highest priority for placement on the OSAC Registry of Standards.

It should be noted that only standards that have been published by an acknowledged Standards Development Organization (SDO) are eligible for publication on the OSAC Registry. While many standards within OSAC disciplines have been published through SDOs, the work of OSAC has revealed that most of these require some modification to reflect the latest advances in forensic science. Likewise, OSAC subcommittees have identified a number of additional standards that have not yet been published through an SDO and, therefore, have begun to develop these standards themselves. Once prepared by an OSAC subcommittee, these documents are passed on to an SDO to ensure transparent input by the broadest possible community of stakeholders. As a result, individuals interested in actively developing standards have at least two opportunities to do so—either through participation in the OSAC or through an SDO. The SDOs with which the OSAC Facial Identification and Speaker Recognition subcommittees are currently engaged include: the American Society for Testing and Materials (ASTM), the Acoustical Society of America, and the American National Standards Institute (ANSI).

In order to facilitate the process by which standards are reviewed and validated through the OSAC, the FSSB has established an ontology to define the categories and subcategories of standards that may be included in the roadmaps. The major categories of standards as defined in this ontology are as follows: Competency; Method Validation; Examination & Analysis; Reporting & Testimony; Quality Assurance; Terminology; and Conclusions, Interpretation, & Opinion. It is expected that individual standards may span more than one of these categories.

During this presentation, attendees will learn about various efforts to develop standards within the disciplines of digital evidence and video/imaging technology and analysis with such topics as: Standard Terminology for Digital and Multimedia Evidence; Examining Magnetic Card Readers; Forensic Audio Laboratory Setup and Maintenance; Data Acquisition from Digital CCTV Systems; Training Guidelines for Video Analysis, Image Analysis & Photography; Latent Print Evidence Imaging Resolution; and Establishing Confidence in Digital and Multimedia Evidence Forensic Results by Error Mitigation Analysis.

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#### Digital Evidence, Forensic Video Analysis, Image Analysis



### C5 The Organization of Scientific Area Committees (OSAC) Digital/Multimedia Scientific Area Committee Standards Work—Part 2: Facial Identification and Speaker Recognition

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**Learning Overview:** After attending this presentation, attendees will be familiar with the latest work in developing OSAC Standards for the Digital & Multimedia Sciences disciplines of Facial Identification and Speaker Recognition.

**Impact on the Forensic Science Community:** Standards are vital to ensuring the sound practice of forensic science across all disciplines. In order to implement these standards, community members must know of their existence and scope. This presentation will impact the forensic science community by offering newcomers an introduction to such standards, while offering more experienced practitioners an opportunity to probe the finer details of the current work and shape the direction of future work.

The OSAC for Forensic Science works to strengthen the nation's use of forensic science by facilitating the development of technically sound forensic science standards and by promoting the adoption of those standards by the forensic science community. These standards are written documents that define minimum requirements, best practices, standard protocols, and other guidance to help ensure that the results of forensic analysis are reliable and reproducible.

The OSAC forensic science disciplines are spread across five major "Scientific Area Committees" or "SACs." The SAC most relevant to the American Academy of Forensic Sciences (AAFS) Digital & Multimedia Sciences Section is the "Digital/Multimedia SAC" (DMSAC). The DMSAC incorporates four subcommittees that address the following disciplines: Digital Evidence, Video/Imaging Technology & Analysis, Facial Identification, and Speaker Recognition. This presentation will focus on the work of the latter two, while a companion presentation addresses the first two.

Recently, the Forensic Science Standards Board (FSSB), which oversees the work of the various SACs and Subcommittees, directed each subcommittee to establish a "roadmap" of standards for their discipline. Each roadmap would identify the individual standards considered to be of the highest priority for placement on the OSAC Registry of Standards.

It should be noted that only standards that have been published by an acknowledged Standards Development Organization (SDO) are eligible for publication on the OSAC Registry. While many standards within the OSAC disciplines have been published through SDOs, the work of the OSAC has revealed that most of these require some modification to reflect the latest advances in forensic science. Likewise, the OSAC subcommittees have identified a number of additional standards that have not yet been published through an SDO and, therefore, have begun to develop these standards themselves. Once prepared by an OSAC subcommittee, these documents are passed on to an SDO to ensure transparent input by the broadest possible community of stakeholders. As a result, individuals interested in actively developing standards have at least two opportunities to do so—either through participation in the OSAC or through an SDO. The SDOs with which the OSAC Facial Identification and Speaker Recognition subcommittees are currently engaged include: the American Society for Testing and Materials (ASTM), the Acoustical Society of America, and the American National Standards Institute (ANSI).

In order to facilitate the process by which standards are reviewed and validated through the OSAC, the FSSB has established an ontology to define the categories and subcategories of standards which may be included in the roadmaps. The major categories of standards as defined in this ontology are as follows: Competency; Method Validation; Examination & Analysis; Reporting & Testimony; Quality Assurance; Terminology; and Conclusions, Interpretation, & Opinion. It is expected that individual standards may span more than one of these categories.

During this presentation, attendees will learn about various efforts to develop standards within the disciplines of facial identification and speaker recognition with such topics as: Training in Facial Identification and Speaker Recognition; One-to-One Facial Comparisons; Collection/Capture of Images for Use in Facial Recognition and Facial Identification; Formalizing the Fundamental Scientific Principles and Vocabulary Inherent in the Speaker Recognition Discipline; Recognizing Foundational Research Publications in Speaker Recognition; Validating Technologies and Methods; Establishing Recommended Procedures for Collection, Processing, and Analysis of Case Evidence; and Determining the Proper Application of Established Methodologies for Highly Varying Case Conditions in Speaker Recognition.

#### Facial Identification, Speaker Recognition, OSAC



## C6 On Generic Digital Forensic Readiness

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**Learning Overview:** The goal of this presentation is to move the discourse on digital forensic readiness from how such information may be collected to a deeper discussion of the cost/benefit trade-offs required (where cost also refers to the privacy of the innocent).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a simple, generic, digital forensic readiness model that allows researchers to propose specific readiness models more concisely. More importantly, the concise description facilitates comparison. In particular, it encourages deeper reflection on the nature, utility, and impact of proactive traces collected.

In digital forensics the phrase “forensic readiness” refers to information that needs to be collected during day-to-day operations of IT systems, such that the evidence required to examine a specific case at some stage will be available and known to be reliable. This presentation provides a generic model of the notion. The intention is to move the discourse from *how* such information may be collected to a deeper discussion of the cost/benefit trade-offs required (where cost also refers to the privacy of the innocent).

Had the phrase “forensic readiness” occurred in other forensic science disciplines, it would probably have referred to the availability of data and samples to facilitate a laboratory’s (or analyst’s) ability to examine a variety of cases. Examples that come to mind are databases of fingerprints, fiber characteristics, and chemical compositions of drugs, to name but a few. The hashes of known software maintained as part of the United States National Software Reference Library is arguably the best-known example of such preparation in the digital forensics discipline.<sup>1</sup>

Note that such “readiness” is not entirely foreign to forensic science. Cockpit voice and data recorders are of immense value when the causes of aviation accidents are examined (and they are present solely for such investigations). In many contexts a (manual or digital) log of activities is maintained that, again, is very useful during an investigation that involves those activities. However, a more abstract (and more formal) description of typical forensic readiness not only serves to better distinguish such work from the examples mentioned above, but also enables one to present a generic forensic readiness model.

In papers on forensic readiness, such readiness is typically engineered for some system  $S$ . The readiness often prepares for some irregularity (such as specific crimes or contraventions of corporate policy). Let  $i$  be some irregular activity (such as spoofing of an email or some specific form of fraud). The set of activities  $A_i$  that would be sufficient to perform  $i$  is then determined. To be ready to examine whether  $i$  occurred, it is posited that each activity in  $A_i$  should leave a trace. Such a trace is recorded in a logging facility, with the nature of the log entry dependent on the information required to prove  $i$ . Let, for any set of traces,  $T$ , the proposition  $\rho(T,i)$  denote that  $T$  is sufficient to prove  $i$ . If  $\rho(T,i)$ , then it follows that  $\rho(T,-i)$ .

This study contends that a simple model based on this notation simplifies the description of a forensic readiness model. The papers explore this for several published readiness models.

Based on the simple model, important questions come to the fore, such as whether the space required for storing traces is warranted by the prevalence (or impact) of any given inappropriate action  $i$ . Questions about the size of the generated traces naturally raise the question of whether the size for any proposed mechanism is minimal. It may be possible to offset the costs of being ready for  $i$  if  $i$ -readiness also implies  $j$ -readiness for an irregular activity  $j$ ; expressed formally, that is when  $\rho(T,i) \Rightarrow \rho(T,j)$ .

A major concern about readiness models is the fact that they collect “evidence” about innocent people even before an irregular activity is performed. It is possible to consider privacy metrics for a set of traces  $T$ . If two equivalent readiness models lead to the collection of traces  $T$  and  $T^0$ , respectively, then the one with the better privacy score is obviously the better choice. However, the nature of such privacy metrics requires further research.

In summary, this presentation presents a simple generic digital forensic readiness model that allows researchers to propose specific readiness models more concisely. More importantly, the concise description facilitates comparison. In particular, does it encourage deeper reflection on the nature, utility, and impact of proactive traces collected?

### Reference(s):

- <sup>1</sup> Rowe, N.C. 2012. Testing the National Software Reference Library. *Digital Investigation* 9:S131–S138. *The Proceedings of the Twelfth Annual DFRWS Conference*.

### Digital Forensic Readiness, Logging, Cost of Extensive Logging



### C7 The National Institute of Standards and Technology (NIST) Scientific Foundation Study for Digital Examiners

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**Learning Overview:** After attending this presentation, attendees will be aware of: (1) the NIST's efforts to develop scientific foundation reviews for various forensic disciplines, and (2) specifically, the development of a scientific foundation study for digital forensics examiners.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the trust in the use of current forensic methods for digital investigations based on solid scientific underpinning and provide a strategic direction for future research in areas that need further strengthening.

"A scientific foundation review is a study that seeks to identify and evaluate the body of scientific evidence that underlies a forensic method or practice".<sup>1</sup> Working with the forensic community, NIST researchers developed a concise method for testing the accuracy of results that could be from a digital forensic examination. This method included collecting metadata related to an examiner's work environment and experience and developing tests targeting digital discovery from hard drives and mobile devices as part of a forensic examination. The goal of this study is to achieve a comprehensive evaluation of outcomes from digital forensic practices used by investigators in both the public and private sector. Protections will be in place to protect the confidentiality and privacy of participants.

To date, there are no major known inter-laboratory studies for digital examiners, prompting the need for this scientific foundational study. This study will attempt to identify differences in work environments (i.e., number of employees, training opportunities, investigator's experience) that could possibly impact laboratories reaching different results. Participants will provide background information describing their current work affiliation, followed by a set of questions related to their acquired skill set, and an electronic discovery test designed for a hard drive or mobile device. The tests are developed with a minimum amount of multiple-choice questions that will focus on forensic artifacts common to any investigation. All responses will be collected in a survey format created for analysis of results. This study is designed as a black box study focusing on the results and not on the methods and practices used by participants to reach their conclusions. Participation in this study is voluntary and open to individuals who conduct digital discovery as part of their official work duties. Upon completion of this study, NIST will publish a report detailing the results.

#### Reference(s):

- <sup>1</sup> John M. Butler, Hari Iyler, Rich Press, Melissa Taylor, Peter M. Vallone, Sheila Willis. *NISTIR8225: NIST Scientific Foundation Review*. September 24, 2018.

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**Foundation Study, Black Box, Inter-Laboratory**



### C8 Attitudes of Citizens of the United States, the United Kingdom, and Turkey in Permits Requested by Mobile Apps

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**Learning Overview:** After attending this presentation, attendees will be able to understand the relationship between risk, privacy, and mobile risk attitudes of citizens of different countries and interpret this in terms of mobile application permissions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees about the difference between attitudes in daily life and the actual situations that occur according to the data security risks that may arise.

How to secure information has become an important problem as application-maker firms have increased their access to personal data. In this context, this research aims to establish the relationship between risk, privacy, and mobile risk attitudes of people living in different countries. For this purpose, a survey of 26 questions was conducted for citizens living in the United States, United Kingdom, and Turkey, provided that there were 150 people from each country (in total 450 people). Surveys of their native languages were conducted for participants in all countries. The survey consists of multiple-choice questions with questions consisting of a Likert scale of 5s.

Hypotheses in the study are “H<sub>0a</sub>: there is no relationship between the risk attitude and the privacy attitude”; “H<sub>0b</sub>: there is no relationship between the privacy attitude and mobile risk attitude”; and “H<sub>0c</sub>: there is no relationship between risk attitude and mobile risk attitude.”

Within the scope of this research, “H<sub>0a</sub>” and “H<sub>0b</sub>” were rejected and a strong positive relationship in the United States, a weak positive relationship in the United Kingdom, and a moderate positive relationship in Turkey were identified. “H<sub>0c</sub>” was also rejected and a moderate positive relationship in the United States and United Kingdom and a weak positive relationship in Turkey were found. Participants were asked which of the nine different types of mobile application permission request options they would not want to grant access to. As a result of the research, the most unwanted permission given in the United States was “location information,” “access to SMS records” in the United Kingdom, and “phone call records” in Turkey.

According to the results of the research, 55.33%, 48%, and 87.39% of respondents in the United States, United Kingdom, and Turkey stated that they are using an Instagram™ app, while, respectively, 54.21%, 68.05%, and 46.56% of users have declared that they will not allow downloads for the application if they see the permissions requested by the application without specifying the application name.

Respectively, 19.33%, 70%, and 95.30% of respondents in the United States, United Kingdom, and Turkey stated that they are using the WhatsApp app, while, respectively, 34.48%, 63.80%, and 46.85% declared that they would not download the app with current permissions.

Respectively, 28.67%, 39.33%, and 22% of respondents in the United States, United Kingdom, and Turkey stated that they are using the Spotify® app, while, respectively, 55.81%, 55.93%, and 42.42% declared that they would not download the app with current permissions. Although the real estate app is different in each country, respectively, 12%, 10%, and 16% of the respondents in the United States, United Kingdom, and Turkey said they are using the real estate application, while, respectively, 50%, 80%, and 70.80% of these users have declared that they will not download the current permissions.

When the results are examined, all hypotheses are rejected for all countries and this means that there was a positive relationship between “risk, privacy, and mobile risk attitude.” In addition, the permissions that the participants do not want to allow are different between the countries. In comparison to the already downloaded applications and declarations against the permits requested without naming the application, it is understood that citizens living in the United Kingdom are more likely to avoid taking risks than citizens living in the United States and Turkey. This has also emerged as an important finding of denial of application permit request at a high rate seen in all countries. Because people avoid reading individual permissions, and because the requested permissions become routine after a certain period of time, differences can arise during the course of the activities with the risk attitudes of people in daily life. This can lead to questions about the sharing and protection of personal information.

#### Mobile Application Permission, Risk Attitude, Data Security



### C9 Apple® iCloud® Message Sync Forensic Investigations and Artifacts

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**Learning Overview:** After attending this presentation, attendees will better understand Apple's® iCloud® Message Sync functionality and its impact on Apple® device forensic examinations of property lists and SQLite databases. Studies of relevant artifacts will be discussed and the changes observed when different user actions occur on an iOS® device in regard to native text messaging and message synchronization to the Apple® iCloud®.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting Apple® iOS® artifact differences relating to user activity surrounding Apple's® iCloud® Message Sync functionality and usage on iOS® devices.

Apple® iCloud® Message Sync has introduced a natively new way for users to sync and store Apple® iMessages®, Short Message Service (SMS), and Multimedia Messaging Service (MMS) (native Apple®) messages. Prior to the introduction of iCloud® Message Sync, an examiner would expect the native Apple® messages to be extracted through an iTunes® backup or using a variety of third-party forensic tools. With the new option for users to store native Apple® messages in their iCloud® account, Apple® no longer needed to include messages in iTunes® and iCloud® backups for disaster recovery or “new device data transfer” purposes.<sup>1</sup> Due to forensic methods and tools relying on iTunes® and iCloud® backup technology to perform extractions, this presented an issue whereby data could potentially be overlooked. This presentation will discuss the forensic analysis of iCloud® message sync artifacts on Apple® iOS® devices that would be potentially relevant to findings in both civil and criminal cases.

Mobile devices, particularly Apple® iOS® devices, have become a common occurrence in both the worlds of digital forensics and e-discovery. Obtaining data from these devices is critical, and oftentimes text messages, such as iMessages®, SMS messages, and MMS messages, contain valuable information relevant to cases. Text messages are typically extracted from Apple® mobile devices through two methods: (1) direct-device collections using a forensic tool or Apple® iTunes®, and (2) analysis of Apple® iTunes® backup files stored via backups to either a local computer or the Apple® iCloud®. In researching these methods, the native Apple® messaging database (sms.db) was collected and parsed by the examiner's tool of choice.

Apple® iCloud® storage services allow users to sync and store data, such as photos, contacts, notes, keychain information, health data, map data, reminders, and more, in their online account. In 2018, Apple® released an iCloud® service called Apple® iCloud® Message Sync, which allowed native Apple® text messages to be stored in a user's iCloud® account and synced to all Apple® iDevices® connected to that iCloud® account.<sup>2</sup> The synchronization of this data from a local device to a user's iCloud® account is controlled from options in the settings of the device. Some of this data, such as photos, notes, and reminders, are set up to sync by default once iCloud® is initially turned on; however, Apple® iCloud® Message Sync data is not turned on by default and must be manually turned on by the user.

The addition of this iCloud® Message Sync service to Apple® devices complicated standard Apple® device collections when native messaging needed to be analyzed. The utilization of iCloud® Message Sync on an Apple® mobile device can lead to the possibility of some data being stored on both the iCloud® and the device or only in the iCloud®. Additionally, when a user has enabled iCloud® Message Sync, traditional iCloud® backups may not contain native Apple® text messages when collected with third-party forensic tools.

Through research, it was able to be determined that the above can affect the conclusions digital examiners make upon collecting text messages from a device. The enabling of the Apple® iCloud® Message Sync option on the device does make changes to both the sms.db database and property list (.plist) files on the device. If examiners do not observe specific data, they may incorrectly conclude it does not exist, when in reality it may be stored solely in the iCloud®.

This presentation will focus on a comparison of Apple® iOS® artifacts to show where examiners can find evidence of iCloud® Message Sync activity. Studies of devices with and without iCloud® message sync turned on will be analyzed and compared, as well as time stamp variances in the sms.db database that may reflect the originating device when multiple iOS® mobile devices are logged into the same iCloud® account. Additionally, a comparison of identified artifact differences in the sms.db tables and property list files will be presented.

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iMessage® Sync, Apple® iOS®, iCloud®



### C10 Joint Task Action Group (JTAG) and Chip-Off Data Analysis and Testing

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**Learning Overview:** After attending this presentation, attendees will better understand the capabilities and limitations of a variety of digital forensic tools that provide support for the analysis of JTAG and Chip-Off binaries from mobile devices operating over the Android™ operating system. The Computer Forensic Tool Testing (CFTT) project at the National Institute of Standards and Technology (NIST) has performed testing across classic digital forensic tools, as well as tools tailored specifically for the data extraction and analysis from mobile devices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview on analysis across multiple JTAG and Chip-Off binaries imported within a variety of digital forensic tools.

As mobile device usage and sophistication continue to grow, the need for rigorous research and testing conducted across a variety of forensic tools and techniques is critical. JTAG and Chip-Off data extraction provide forensic examiners with the ability to often recover additional data in comparison to a logical or file system data extraction. JTAG is a non-destructive method that returns a byte-for-byte memory dump of accessible data from supported mobile devices. Chip-Off is a destructive technique that entails removing the flash memory chip from the Printed Circuit Board (PCB). Removing the flash memory entails cutting and grinding the PCB, allowing the chip contacts to be exposed. Once the chip has been prepared, the memory registers of the chip are read utilizing the correct adapter and by running a programmer application.

The JTAG and Chip-Off research and testing conducted within the CFTT lab includes JTAG and Chip-off binaries from a variety of mobile devices. Each mobile device was populated with a defined dataset, including active and deleted data across numerous types of data elements. In addition to binaries collected using either the JTAG or Chip-Off data extraction technique, data for supported devices were extracted using both JTAG and Chip-Off. Analysis across multiple devices and techniques provides insight into advantages of one technique versus another. Additionally, performing both data extractions on supported devices illustrates any differences between JTAG and Chip-Off extractions for a unique mobile device.

The goal of this research and testing within the CFTT program is aimed at providing the forensic community with an understanding of the capabilities and limitations of various digital forensic tools that support analysis of JTAG and Chip-Off binary files. These results provide insight into any pros and cons across a combination of supported techniques and tools.

The presentation gives a summary of findings and lessons learned during the research and testing process of tools capable of extracting and analyzing memory contents from numerous JTAG and Chip-Off binaries.

*Certain trade names and company products are mentioned in the text or identified. In no case does such identification imply recommendation or endorsement by the author or the author's employer, nor does it imply that the products are necessarily the best available for the purpose.*

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#### Mobile Forensics, JTAG, Chip-Off





### C11 Forensic Gait Analysis: A Strength of Evidence Evaluation From Closed-Circuit Television (CCTV) Footage

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**Learning Overview:** After attending this presentation, attendees will better understand the application of a morphometric technique for analysis and the subsequent approach to evaluate the strength of the evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by revealing a gap within research that was addressed via a method of evaluating forensic gait examination through assessing the strength of evidence that was successfully applied to the research, thus highlighting both competence and performance.

**Introduction:** The ubiquitous nature of surveillance cameras allows continuous monitoring of an area where footage can be obtained for later use, if criminal or other activity of interest occurs, for investigative and evaluative purposes. In the process, gait is often important, as facial analysis is not always possible due to obstruction of the face. Subsequently, a photo-comparative analysis of the footage and of a Person of Interest (POI) may be required. Such examination involves evaluation of the strength of evidence at both activity (relates to activity undertaken by the individual within the footage) and source level (alludes to features observed within the anatomy and gait of an individual), thus underlining its importance.

**Goals:** The goals of this research are to develop a standardized method for morphometric body and gait analysis that incorporates the quantification of image distortion and to determine distinct features of the body during gait (stance, walk, run).

**Hypothesis and Specific Objectives:** The hypothesis and specific objectives include the development of an analytical model that shows distinctive features of gait in a forensic context while determining features of the body during stance and gait (walk and run) and determining the frequency, distinguishability, and dependency of the features within subpopulations while viewing correlations of age/ethnicity/sex and examining the robustness of gait to different conditions (person, accessories, and environment) observed in forensic scenarios.

**Method:** The method includes a morphometric assessment of 18 anthropometric measurements (static, dynamic, and angle), 25 morphological features for stance, and 52 morphological features for gait of both male and female volunteers.

**Results:** As a result, a standardized protocol was produced, and population databases established from which frequency statistics are attained. Moreover, features were observed as either common or distinct, once compared to all age, race, and sex categories for correlation determination. These components were then applied to assess the strength of evidence between the trace and the reference materials, resulting in a likelihood ratio value.

**Conclusion:** As a forensic tool, the forensic gait analysis method often lacks validation, and its evaluation misses empirical substantiation. Nevertheless, the availability of trace material in numerous cases and the potential for development of the method suggests that research on this topic cannot be overlooked. The broader purpose of this study established a method of evaluating gait analysis that offers valuable information to the criminal justice system while being scientifically robust and highlighting its limitations.

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**Forensic Gait Analysis, CCTV, Morphometric**



### **C12 An Acceptable Resolution for the Identification of Knives Captured Within Closed-Circuit Television (CCTV) Imagery**

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**Learning Overview:** After attending this presentation, attendees will better understand the reliability issues surrounding the identification of knives within CCTV imagery and will be presented with findings from research into this area.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sharing research pertaining to the minimum acceptable resolution for the analysis of a purported knife within CCTV imagery, thus increasing reliability of said analysis.

Previous research has been conducted to define thresholds for the face in terms of facial width in resolution and pixels as well as bit-rates, and it is the recent spike in knife crimes within the United Kingdom that has driven the need for research into these measurements in relation to knives.

The increase in knife crime over the past few years within the United Kingdom has led to a substantial increase in the purported identification of knives from CCTV imagery to provide evidential support in criminal trials. As no weapon is recovered in the majority of cases, a precedent appears to have developed for both forensic image examiners and police officers to render opinions such as “appears to be a knife as it is shiny” without the application of any form of methodology. It is also common for those accused to claim the object is an e-cigarette, further confusing the analysis. Imagery from real casework will be shared to allow attendees to gain an understanding of the issue and why research into this area is essential.

The ability to render an opinion, and the reliability of such, is in direct correlation with the quality of the imagery and the features that an examiner believes are consistent with an object.

To address the above, the research presented tests the following hypothesis: the minimum dimensions required for a reliable opinion to be given in relation to an object purported to be a knife can be defined.

To test the proposed hypothesis, CCTV imagery of both knives and e-cigarettes of similar dimensions, colors, and materials were captured at decreasing resolutions in conditions mimicking real casework. These included differing encoding algorithms, environments, perspectives, and lighting. Analysis was then performed using a morphological analysis methodology until it was deemed of insufficient quality to draw a reliable opinion. Attendees will be presented with the detailed methodology and subsequent findings.

It is of the utmost importance that analysis of objects purported to be knives is as reliable as possible, since in a large number of these cases, the defendant is facing extended periods of imprisonment, especially if the alleged crime is murder. The research presented aims to go some way in increasing the reliability of any analysis performed in relation to such through an initial analysis suitability triage through the use of an acceptable object resolution threshold that can be utilized in casework.

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**Imagery Analysis, Video Forensics, Weapon Analysis**



### C13 Unlocking Apple® Mobile Devices: A Forensic Practitioner's Perspective and Lessons Learned

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the current capabilities of GrayKey as it applies to specific case examples at the Defense Forensic Science Center (DFSC).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the benefits and limitations of GrayKey as it pertains to the unlocking and extraction of data from mobile devices in a digital forensics laboratory.

Smart phones are becoming increasingly important in digital evidence investigations. Obtaining data from a smart phone may assist in cases dealing with any number of criminal activities, including theft, sexual assault, and homicide. Evidence extracted from mobile devices may include pictures, call logs, text messages, e-mails, videos, and documents. While this evidence may be critical to a case, accessing the information may prove difficult, especially if the device is locked with enhanced security methods (Personal Identification Number [PIN]/passcode lock, pattern lock, fingerprint, facial recognition, etc.) or utilizes data encryption.

The Apple® iPhone® is one of the most popular smart phones within the United States. Bypassing the security features of Apple® mobile devices to gain access to any maintained data is of great importance to law enforcement and digital forensic examiners. One primary tool used for gaining access to locked Apple® mobile devices is the topic of this presentation.

The tool is a device that allows users to attempt to access locked Apple® iOS® devices within their own facilities. Apple® mobile devices are connected one at a time to the unit, which uses proprietary software to gain access to the device. Once access is gained, the tool performs two actions. First, software designed to determine the unknown passcode is introduced to the mobile device. Second, data is extracted from the device, with accessibility to certain types of data dependent on the security status of the device. When an iOS® device is first powered on, it is in Before First Unlock (BFU) mode. Once the passcode has been entered on the device, it switches into After First Unlock (AFU) mode. Prior to determining the passcode of the mobile device, the tool can perform both BFU and AFU partial file system extractions. After the unknown passcode is determined, the full contents of the file system may be obtained.

The tool has been used in numerous cases at the DFSC since the fall of 2018. In addition to completing routine data extractions from mobile devices, many lessons and unique scenarios have been encountered since the introduction of this capability at the DFSC. During this presentation, insight will be given into the types of scenarios that have been encountered, as well how obstacles have been addressed. Several unique case examples will be highlighted by both an experienced digital forensic examiner and an examiner new to mobile forensics. The experiences and lessons learned will demonstrate how the tool has been implemented in a forensic laboratory setting.

*The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army (DA) or the Department of Defense (DoD). Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors, DFSC, United States Army Criminal Investigation Command, Office of the Provost Marshal General, DA, or DoD.*

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#### Mobile Device Forensics, Cell Phone Forensics, Digital Extraction



### C14 2D/3D Imaging for Forensic Ballistics Comparison Assessment

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**Learning Overview:** After attending this presentation, attendees will be familiar with various comparison techniques used in the field of forensics firearm examination that are currently based on analysis of 2D and 3D imaging of cartridge cases and bullets. This presentation will attempt to standardize all the existing solutions by presenting a simple but schematic view. All the techniques will be presented, organized in various categories, and the pros and cons will be exposed considering the practical application scenarios.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing attention to innovative techniques in support of forensic ballistics analysts.

The main steps of a firing process of a self-automatic gun are: loading a round of ammunition into the chamber, shooting the bullet through the barrel, and ejecting the spent cartridge case. During this process, the class and individual characteristics of the gun are transferred from the hard surface of the weapon to the softer surface of the bullets and cartridge cases as a unique fingerprint. When a firearm is used to perpetrate a crime, bullets and cartridge cases will be found in the collected evidence on the scene; it is possible to then identify both the class (make and model) and the individual weapon that was fired. Currently, the collected bullets and cartridge cases on the crime scene are analyzed using an optical comparison microscope. The main limitation of this method is that the overall forensic evaluation made by experts, in some cases, could make it difficult to report the value of the scientific findings in terms of balance, logic, robustness, and transparency.<sup>1</sup>

The possibility of representing cartridge cases in 2D images, topography images, or in 3D point clouds has opened up new research challenges as well as the opportunity to move from manual optical comparison techniques to more sophisticated semi-automatic digital techniques, with the aim of obtaining objective assessments.

In 2015, the National Institute of Standards and Technology (NIST) standardized the forensic ballistics comparison analysis by introducing the Congruent Matching Cells (CMC).<sup>2</sup> Exploiting this method, based on the principle of discretization of cartridge case images, it is possible to obtain an accurate identification process and, consequently, an estimation of the error. In late 2016, the NIST Ballistics Toolmark Research Database (NBTRD) was published as an open-access dataset containing 2D and 3D acquisitions of bullets and cartridge cases fired by different guns.<sup>3</sup>

The ballistic comparison based on 2D images is very sensitive to lighting conditions and, consequently, some important details for the comparisons could be lost. These anomalies do not occur if one considers a representation of cartridge cases and/or bullets as point clouds in 3D space. In this way, it is possible to reconstruct the geometrical structure of the element itself and obtain a more accurate ballistic comparison.

This presentation will show techniques based on 2D images up to those that work on 3D point clouds.

Taking advantage of 3D representations of cartridge cases, in recent years, several very useful solutions have been implemented to obtain an accurate result. Giudice presents a technique that identifies the primary keypoints in order to perform a good alignment operation between the 3D point clouds.<sup>4</sup> Excellent results have been obtained exploiting the neural networks as described in Giudice et al.<sup>5</sup> By means of Siamese Neural Network, the distance between point clouds in 3D space is calculated for classification tasks (type and gun identification). Finally, a new “immersive 3D technology” will be presented, aimed at supporting the operator during the analysis phase.

#### Reference(s):

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2. Song, John. Proposed “congruent matching cells (CMC)” method for ballistic identification and error rate estimation. *AFTE J* 47.3 (2015): 177-185.
3. Zheng, Xiaoyu A., Johannes A. Soons, and Robert M. Thompson. *NIST Ballistics Toolmark Research Database*. No. Forensics@ NIST 2016. 2016.
4. Giudice, Oliver. *Digital Forensics Ballistics: Reconstructing the source of an evidence exploiting multimedia data*. (2017), PhD thesis, University of Catania.
5. Oliver Giudice, Luca Guarnera, Antonino Barbaro Paratore, Giovanni Maria Farinella, Sebastiano Battiato (2019). *Siamese Ballistics Neural Network*. 26<sup>th</sup> IEEE International Conference on Image Processing (ICIP), 2019.

#### Firearm Recognition, 3D Data, Ballistic Comparison

### C15 A Morphometric Analysis of Ears in Twins: An Aid to Forensic Personal Identification

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**Learning Overview:** After attending this presentation, attendees will better understand the uniqueness of morphological and biometric features of ears from one individual to another and in the same subject.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the importance of evaluating morphological characteristics and dimensions of the auricle as a tool for forensic identification of living or deceased individuals.

The idea of using ears for identification purposes dates back to the late 19<sup>th</sup> century when Bertillon included it as one of 11 anthropometric measurements in his manual system for identifying individuals.<sup>1</sup> In fact, although the primary function of the pinna is to collect sound waves and transmit them to ear drum through the external auditory meatus, the ear is an under-recognized defining feature of the physiognomy of the face.<sup>2</sup>

Since the morphological characteristics of the external ear stay fixed in the same person after completion in development until death, while they vary from one individual to another according to age, sex, and ethnicity, comparative analysis of ear biometrics represents a useful tool in forensics for positive identification of living or deceased people.<sup>2,3</sup> As the morphology of ears tends to be hereditary, the present twin study was undertaken to determine morphological and biometric variations of both ears within pairs for individualization purposes.<sup>4</sup>

The study was conducted on four pairs of homozygotic twins: two males and six females, aged 18 to 45 years. Each pair underwent facial 3D laser scanning using a Head and Face Color 3D Scanner, with the subjects' heads in Frankfurt Horizontal plane. Acquired facial surface reconstructions were obtained through Cyberware Echo software. Reference vertical (Y, midline through glabella) and horizontal (X, through right and left endocanthion) planes were constructed on the models. For each patient, the following standardized measurements of both ears were recorded twice by a single investigator using landmark points defined by Farkas and colleagues: Total Ear Length (TEL), Ear Breadth (EB), Distance Preaurale-Glabella (P-G), Preaurale-Subnasion (P-Se), Preaurale-Exocanthion (P-Ex), and Preaurale-Pogonion (P-Pg).<sup>5</sup> The Ear Index (TEL/EB X100) was calculated for both ears. Morphological ear shapes and lobule attachment were also noted. Intra-individual and intra-pair comparisons of those parameters were conducted. Data were analyzed using an Excel<sup>®</sup> statistical analysis program.

When comparing both sides in the same individual, complete symmetry was noted regarding the shape of the auricles and lobule attachment, with 100% intra-pair concordance. Triangular shape was the most common (50%), followed by the oval (25%) and round ones (25%). The lobule was attached in 25% of individuals, while it was free in 75% of individuals.

The mean value of TEL was 60.8±9.4mm (right ear R: 61.14±9.31mm; left ear L: 60.46±10.13mm). An average Intra-Individual Discrepancy (IID) of 2±1.72mm was found comparing R and L. A mean Intra-Pair Discrepancy (IPD) of 2.19±1.85mm for R and of 1.59±1.63mm for L was noted. The mean value EB was 33.79±4.35mm (R: 34.28±4.43mm; L: 33.3±4.51mm). The mean IID of breadth was 1.32±0.63mm and IPD of 0.85±0.63mm in R and of 0.96±1.03mm in L was noted. The mean total Ear index was 55.57% (R: 56.08% and L: 55.08%) with a mean IPD of 2.97% and a mean IID of 0.63% and of 2.73% for R and L, respectively. All ear anthropometric dimensions were found to be significantly higher in males. TEL and P-Ex distance were the only two parameters showing higher values on L. As far as EB and P-Pg distance were concerned, there seemed to be a higher variability when comparing the two ears of the same individual than when considering the auricles on the same side of the twins in each pair. On the other hand, TEL, P-G, and P-Se showed higher IPDs, especially when considering the right ears. P-Ex was the only parameter showing comparable IID and IPD.

The results of this pilot study on variations of ears morphology between homozygous twins can be used as supportive evidence that ear biometrics could represent a helpful tool in forensic issues since auricles showed measurable variations even between individuals sharing the same genetic heritage.

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#### Homozygous Twins, Personal Identification, External Ears Biometrics



### C16 The Development of a Cleaning Protocol for Mobile Devices Contaminated With Controlled Substances

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**Learning Overview:** After attending this presentation, attendees will understand the dangers digital forensics analysts face with regard to drug exposure and will learn decontamination protocols that will protect analysts while allowing for digital data acquisition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing protocols that support both the digital forensic analyst's safety and digital data acquisition when cleaning digital devices contaminated with controlled substances. The focus of this presentation is on the decontamination of mobile devices from various illegal and potentially dangerous drugs, such as heroin, cocaine, methamphetamine, and fentanyl/carfentanil. In addition, this presentation will recommend appropriate Personal Protective Equipment (PPE) that is vital to the safety of the digital analysts.

Digital evidence submitted with potentially undetected dangerous illicit substances has become a huge concern in the digital forensics community. While there are suggested protocols and best practices to collect liquid, structurally, and thermally damaged devices, there is little-to-no accepted protocol within the forensics community to effectively clean drug-contaminated devices that ensures both the safety of the analyst and a complete data acquisition from the mobile device.<sup>1</sup>

A set of 60 Blackberry® 8330 Curves and Blackberry® 9330 Curve 3s were utilized for this research. Initial hash values and total size from file system and physical acquisitions were obtained using Cellebrite® UFED 4PC version 7.18.0.199 for all of the devices used. Both industrial and household cleaners were used as decontamination cleaners. The industrial cleaners tested include: Apple® Environmental® Meth Remover Parts 1 and 2, First Line Technology® FiberTect Polyester Pads/Wipes, First Line Technology® Dahlgren Decon 200mL Ready-to Use Configuration, and Intelgard® EasyDECON Parts 1 and 2. The household cleaners included Lysol® Fresh & Clean Multi-Surface Cleaner, Formula 409® Multi-Surface Cleaner, Alconox, Inc.® Liquinox 1232 Critical-Cleaning Liquid Detergent, Fiberlock Technologies® Shockwave-Concentrate, and Scrubbing Bubbles®. Each controlled substance was diluted in methanol and run on a Gas Chromatograph/Mass Spectrometer (GC/MS) equipped with a Restek® DB-5 column (30.0m x 250µm id x 0.25µm particle size) to obtain reference data. The devices were then systematically exposed to each drug, and the presence of the drug was confirmed using the GC/MS. Each cleaner was used to attempt to clean the controlled substance from the device to the point of no detection. Cleaning steps were repeated if the drug remained detectable.

Data acquisitions from each digital device were obtained both immediately after the cleaning process and one week after to simulate the environment of a working digital forensics laboratory. Advanced data extraction techniques such as chip-off or JTAG were attempted if a traditional cable acquisition was unsuccessful. The data acquired after cleaning was compared to the initial hash values and flash.bin file sizes. The success of each data acquisition was then used to create a list of recommended decontamination methods for digital devices contaminated with the tested controlled substances.

#### Reference(s):

<sup>1</sup>. SWGDE Best Practices for Collection of Damaged Mobile Devices. *Scientific Working Group on Digital Evidence*, Version 1.1. 2019.

#### Damaged Devices, Controlled Substances, Cleaning Protocols



### C17 The Application of Artificial Intelligence (AI) in Digital Forensic Science

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**Learning Overview:** The goal of this presentation is to learn from different possibilities in AI and know the limitations and possibilities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a brief statement on how AI impacts digital forensic science; however, validation is always needed.

AI tools that work like humans and learn from examples are often integrated into many different software tools, such as object detection tools, Automated Fingerprint Identification Systems (AFIS), face recognition, speaker recognition, author recognition, and many more. We see many different tools that are combined and used in pipelines to extract evidence from big data.

AI is included in many search engines and many products, and we are used to having them. Now, smart phones often include much AI for recognizing the user and predicting the information someone is interested in. Speech recognition works well enough to use for searching the internet. People are sometimes not aware that their digital devices, such as tablets and smart phones, are listening to their conversation if they have not turned the privacy modes on. Later, the user might get advertisements related to some words they were discussing, such as, for example, a vacation to Corsica if they were discussing that part of the world. In criminal settings, using certain phrases may later be used as supporting evidence in court.

For facial comparison, it is well known that bias exists, depending on the training sets. There are cities, such as San Francisco, that abandoned the use of this technology by the government due to bias and other privacy concerns. However, since facial comparison algorithms have proven to be much better, and in many cases outperform humans, the use of an algorithm to assist the forensic scientist is used more often. Sometimes, we have patterns, such as the Photo Response Uniformity (PRNU) to identify cameras, that a user cannot really see, and an algorithm can compare better. In practice, we see that AI is used to make faces that never existed, based on training material, such as the deep fakes.

For image and video manipulation detection, more algorithms are developed that also use deep learning techniques to detect the manipulation. It is very important that we can explain in court how these algorithms work, and that the results are validated. Also, we need to be aware that once a method for detection has been published, it is possible to attack the detection method. For this reason, it is necessary to perform continuing research on the application of methods in AI, without seeing it as a complete black box. The reproducibility of results with AI is another issue that should be addressed, since based on the starting point of a dataset, different results may be obtained.

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**AI, Neural Networks, Digital Forensic Science**



### C18 Cloth Classification With a Semi-Supervised Generative Adversarial Network (SGAN)

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**Learning Overview:** After attending this presentation, attendees will be aware of new possibilities for tracking persons based on a description of clothing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing novel ways of searching for certain clothing items on video based on machine learning.

If large amounts of video are collected from different camera systems, often it is necessary to track a person based on clothing, especially if the face is not visible in the images. With that information, investigators can look for the person on Closed-Circuit Television (CCTV) and recordings from other sources in the relevant area. This is a simple task in theory, but tedious in practice, since there may be many hours of video footage with a high number of people in them. The goal of this presentation is to automate this task using machine learning methods.

One practical obstacle to achieving this goal is a lack of labeled training data. A semi-supervised learning method was applied to circumvent the lack of relevant, available datasets. A semi-supervised SGAN was used. This SGAN consists of two networks, a generator, which generates images, and a discriminator, which classifies images into the different classes or as being a generated image. The generator receives feedback from the discriminator, and will thus create more real looking images, and the discriminator will learn to correctly classify the images. The idea is that the discriminator will also learn from the generated images, even though they are not labeled as one of the classes.

New annotations were provided for 280 images of an existing dataset of surveillance footage (CUHK Person Re-identification Data Set). These annotations consist of manually tagged clothing items. New images were created, where each image contained only one clothing item. This was to simplify the task, instead of making it a multi-label classification problem. The ten classes consisting of the most images were selected. These were: a backpack, a bag, boots, a coat, jeans, oxfords, sneakers, a sweater, trousers, and a T-shirt. The SGAN was trained using 25, 50, or 100 images per class. The performance of the SGAN was compared to that of a baseline model, where the generator never got updated, so the generated images do not resemble the real image. As expected, the baseline performed better the more images it had to train with. However, the SGAN performed worse than the baseline, regardless of the amount of images it had to train with. Visual inspection of the generated images showed that these did not look like the real images; this could be an explanation for the worse performance of the SGAN. With the SGAN, training on 100 images per class, with ten classes, an accuracy of 64% was achieved. This paves the way to classification of clothing in the field of multimedia forensics.

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**Deep Learning, SGAN, Clothes**





### C19 A Wild Manhunt for Stego Images Created by Mobile Apps

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**Learning Overview:** The goal of this presentation is to describe the challenge of detecting images with secretly embedded information by mobile apps. This presentation will evaluate academic algorithms and commercial software for this problem and make great progress with the proposed methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the realization of the situation of wide-spread usage of stego apps for illegal purposes and the possibility of detecting them. This presentation will also introduce some fast and high-accuracy tools aimed at some specific photo editing apps.

Digital image forensics is a young but maturing field, encompassing areas such as camera identification, forgery detection, and steganalysis. Steganalysis is the analysis of image data to discover if hidden content is contained within the image, and, if so, to uncover further information about the hidden content. Most of the academic research on steganalysis has focused on identifying or classifying an image as cover (innocent) or stego (with hidden content). However, large gaps exist between academic results and applications used by practicing forensic analysts.

To move academic discoveries closer to real-world implementations, it is necessary to use data that represent “in the wild” scenarios. This project looks at stego images created by mobile apps. For the past three years, research has been conducted on collecting stego images from different phones and different stego apps. One of the main contributions is a procedure for generating a large image database by using Android® emulators and reverse engineering techniques. In 2019, StegoAppDB, the first database consisting of stego images produced from mobile apps, was built and put online.<sup>1</sup> With the large amount of data from the StegoAppDB, for the first time, the performance of software that is designed for stego detection can be tested. Although most stego apps implement some classical embedding algorithms, none of those commercial programs have successfully detected images from apps. One main reason is that those old detection programs rely on the hidden fixed patterns, such as signatures or watermarking, for the algorithm developers, which are completely erased by the app developers.

In the December of 2019, another work was presented to discuss steg detection on images from mobile apps by using two different approaches: “signature” detection and Machine Learning (ML) methods.<sup>2</sup> This study analyzed Android® apps that implement steganography algorithms by applying reverse-engineering techniques to the binary code. In analyzing the code, it was determined that most algorithms used to hide the message are far from the advanced algorithms published in academic research papers. Some apps provide little security, even if a complicated embedding method was used but strangely had a unique “signature” embedded, and make the stego image and its app easily identifiable as such. Moreover, the extraction of hidden information for those app can be achieved. This study developed detection tools for all images with well-defined “signature” patterns and achieved nearly 100% accuracy. For the apps that do not have the “signature”, the study applied the ML-based detection methods to identify stego images. ML has been widely used in the academic community. However, applying those ML algorithms to the real-world data is not trivial. First, the real-world data has more variance in exposure settings and embedding rates, which is not the typical case in the academic world. Second, an input image provided by the user is processed before the embedding step by the app, and, therefore, an input image is not necessarily the cover image in the traditional definition. This study summarized most pre-processing methods and output the post-processed images as cover images for training the classifiers. This study used instrumentation techniques to perform the non-trivial task to batch-generate cover-stego image pairs for ML steganalysis. These proposed ML methods can detect both spatial domain and JPEG domain stego images with a decent accuracy. Per research, this is the first time an ML detection algorithm was applied to identify stego images generated by mobile stego apps.

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- <sup>2</sup> W. Chen, L. Lin, M. Wu, and J. Newman: “Tackling Android Stego Apps in the Wild,” IEEE APSIPA Annual Summit and Conference, Hawaii, 2018.

#### Digital Image Forensics, Steganalysis, Android™ Apps



### C20 Control Point Selection for Analysis and Height Measurement From Closed-Circuit Television (CCTV) Images

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**Learning Overview:** The goal of this presentation is to illustrate the effects of control point selection on precision and accuracy in the analysis of CCTV footage. The hypothesis of precise and accurate measurements in the absence of control points is also presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining that validation of the control point selection process and parameters, such as camera resolution, is an important step in presenting photogrammetric evidence with known error rates. The hypothesis that natural image landmarks can be just as robust when substituted in the place of purpose-built control point markers may significantly reduce the time and resources police are required to spend in the field, and it may enhance the analysis of CCTV evidence where measurements are involved by improving the calibration process.

Agencies may use CCTV footage to extract meaningful information about the movements, activities, and characteristics of a person of interest. One such characteristic is height, and as a class characteristic it is a powerful exclusionary tool. In addition to the capabilities of laser scanning and photogrammetry to create 3D photo-realistic reconstructions of crime scenes, there is keen interest in applying these techniques to ascertaining height measurements from recorded traces left on CCTV footage. Control point selection has shown promise in a number of studies focused on obtaining height from single-image surveillance video. Control point selection as a photogrammetric tool in crime scenes currently has limited literature—especially when it comes to establishing precision and accuracy and assessing the impact of image variables on precision and accuracy.

In this work, experiments were designed to test the variability of photogrammetric errors with image resolution. A calibration set-up with 50 purpose-made control point targets was filmed and scanned. Using a photogrammetric package, control point selection was used to achieve camera calibration, followed by derived height measurement of a physical object of known height placed in the field of view of the footage. The resolution of the original footage was digitally down-sampled in 5% increments and the measurements repeated. It was hypothesized that image resolution would be inversely proportional to precision, with an unknown effect on accuracy. The effect of uneven control point distribution within the space was also investigated. The accuracy of the measurements was externally validated using Light Detection And Ranging (LIDAR).

It was found that image resolution did systematically impact the precision of the results for height measurement and calculation of the camera location. It was observed that the greatest error range in calculated camera position and measured height was in the lowest-resolution image.

It was found that the accuracy was impacted by the nominated location of the control point in the image, which is a manual and subjective process. Concern was raised as to subjectivity of manual point selection, and the question is posed as to whether relying on automated target detection or shape-matching algorithms (which are already commercially available), would be a better option.

Follow-up experiments were conducted to investigate whether purpose-made control points could be replaced by immovable existing features within the field of view of the camera, an area of research that has limited/no existing literature. Precision and accuracy analysis using landmarks was conducted in a similar manner to the earlier height measurement, noting that existing fixed features may not be evenly distributed in the field of view.

#### Photogrammetry, Control Points, CCTV



### C21 Using an Orthogonal Fingerprint Matcher to Boost Recognition of Contactless Fingerprints

Mark A. Walch, MArch, MPH\*, *Sciometrics LLC, Chantilly, VA 22150*

**Learning Overview:** After attending this presentation, attendees will better understand the capabilities and possibilities as well as the current status of off-the-shelf smart phone technology as a fingerprint-capture device in support of identity matching. Technical details will be provided regarding the process of capturing accurate photographs with a smart phone camera, rendering these images into high contrast fingerprints and automatically matching these fingerprints to identities. A new concept will be presented that contactless fingerprints should be handled as latent fingerprints for matching purposes with final adjudication of results performed by an orthogonal matcher.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing a new idea that contactless fingerprints be treated as latent prints and discussing the steps for implementing this concept. Specific reporting will be provided on the development and testing of techniques for capturing contactless prints using a mobile device and submitting these prints to an Automated Fingerprint Identification System (AFIS) as latents against legacy “rolled” or “flat” prints. Once the AFIS results are returned, this presentation will discuss the adjudication of the matching results using orthogonal recognition technology originally developed by the Federal Bureau of Investigation (FBI) Laboratory (Project GRAFF) as an “Afterburner” and generating an objective score that makes a statistical statement regarding the results.

Fingerprints are truly the “human barcode” and among the best measures of human identity available. Conventional fingerprint sensors require a person to touch the device platen or sensor. Disadvantages to this mode of acquisition include the time required to collect (particularly rolled) prints, as well as hygiene concerns. Recently, technologies have been developed to use smart phones as fingerprinting devices. Since capturing fingerprints with the camera on a phone does not require physical contact, this method of collection has been labeled “contactless fingerprinting.” Contactless fingerprinting can be performed by the “rear” phone camera with no additional hardware. A 12-megapixel camera can produce high-resolution images (from 500 to 1,000ppi) that capture sufficient ridge detail to support fingerprint matching. A typical strategy for contactless fingerprinting is to capture ten fingers in three pictures: two “slaps” (four fingers each) plus two thumbs held together. Once captured, the images are processed into high-contrast prints; features are extracted from these prints and placed into an AFIS query format. Matching can either be performed on the mobile device or the fingerprint images can be set to a remote server for matching.

Images produced by contactless devices are fundamentally different from conventional scanned ink and livescan fingerprints, supporting the concept that they be treated as latents. Contactless and contact prints differ in both distortion characteristics and image sensor characteristics. New pathways for device certification are being developed for contactless fingerprinting to ensure these new contactless fingerprint images are matchable to conventional fingerprints captured through inking or contact scanning. For example, through funding from the FBI’s Biometric Center for Excellence, the National Institute of Standards and Technology (NIST) has been developing standards for certifying contactless scanning so images obtained in this manner can be submitted for matching against the FBI’s databases. However, the body of current research focuses on what can be done on the mobile device to create contactless images that are most compatible with legacy collections. This presentation discusses a different approach in terms of technology that can be employed to handle the contactless fingerprint transaction. The content of the presentation focuses on a specific approach for submitting limited sets of prints (such as would be the case for latents) and introducing orthogonal matching technology that will modify the matching process without fundamentally changing the nature of how existing AFIS systems work. The outcome of the modified process is improved performance for matching contactless prints submitted against collections of prints obtained through contact methods.

This presentation of treating contactless prints as latents will be supported by actual data obtained through contact fingerprinting and matched against an AFIS with contact reference prints taken from the same and different individuals. These results will show original accuracy scores for contactless images against legacy contact prints using a commercial AFIS. In contrast to these original scores, results incorporating the orthogonal technology as an Afterburner are also shown to demonstrate its impact on scoring and ensuring data from all ten fingers can be reliably collected using contactless methods.

#### Contactless, Fingerprints, Orthogonal



### C22 Machine Learning in Data From Crime-Related Mobile Devices: Bidirectional Recurrent Neural Networks for Named-Entity Recognition (NER) in WhatsApp, Instagram™, and Facebook® Messenger Text Conversations

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**Learning Overview:** This presentation summarizes the initial efforts of a Brazilian Federal District Police forensic research team to use the huge amount of text data extracted from crime-related mobile devices to shed light on the general crime panorama of the Brazilian Federal District and to develop tools that can help the digital forensic investigator in his casework. Attendees will have a chance to see the nature and format of the text data extracted from mobile devices in Brazil, the path chosen by the research team to retrieve drug-related information from this data, the difficulties encountered, and the solutions developed by the team.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing how recently developed Bidirectional Long Short-Term Memory (LSTM) Neural Networks can be used to extract information from text data retrieved from crime-related mobile devices. This presentation will show the strategy developed by the forensic research team to perform drug-related named-entity recognition on WhatsApp, Instagram™, and Facebook® Messenger Text Conversations.

It is notable that in recent years there was a significant increase in the volume and variety of data in the hands of corporations and institutions. Most of this data is represented in the form of unstructured text. Many are examples situations in which huge quantities of text data can be used to achieve valuable insight.

The Digital Forensics Department of the Brazilian Federal District Police works as a hub to a huge amount of data retrieved from mobile devices owned by crime perpetrators and victims. This data group represents an untapped potential. The correct interpretation of this data can bring valuable information on the general panorama of criminal offenses in the Federal District.

In order to start exploring this data group, the forensic research team considered the development of a tool to extract drug-related information from text conversations. Machine learning techniques have already been successfully employed to identify crime-related images on police databases, but content-sensitive text analyses are yet to be used in this context.<sup>1</sup>

NER is an information extraction technique that aims at locating and classifying named-entity mentions in unstructured text into predefined categories, such as person names, organizations, quantities, etc. Recently published research used a Recurrent Neural Network to perform NER in Brazilian legal texts, obtaining satisfactory results.<sup>2</sup>

A Bidirectional LSTM Neural Networks was chosen to perform drug-related NER in text conversations retrieved from mobile devices seized by police officers in the Federal District. The researchers identified a similarity between these text messages and tweets made by Brazilian users, so public tweets were used as a complementary source to the database.

After a pre-processing stage, the database was manually labeled. The entities were assigned the values B-DRUG, for terms that represent names of drugs, and B-DRUG\_RELATED, for terms that represent activities related to drug abuse. The final base was made up of 102,325 words, of which 7,356 refer to drugs and 2,148 correspond to drug-related activities. After the construction of the database, a Bidirectional LSTM Neural Network was implemented and trained with the database.

The model was then tested. The base was randomly divided, using 80% of its data for training, and 20% for testing. Experiments revealed that the model was able to satisfactorily classify the test data, obtaining a precision rating of 0.99 for B-DRUG terms and 0.97 for B-DRUG\_RELATED terms. The F1-Scores were 0.99 and 0.98, respectively. A validation test was performed using only data from the mobile devices, resulting in a precision rating of 0.91 for B-DRUG terms and 1.00 for B-DRUG\_RELATED terms. The F1-Scores were 0.74 and 0.93, respectively. These results are promising. Content-sensitive text classification in text messages is a difficult task, so named-entity recognition can be used in machine learning applications to aid in the fight against crime.

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#### Machine Learning, Named-Entity Recognition, Text Analytics



### C23 Steganography Detection Using Machine Learning (ML)

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of steganography, steganalysis, and ML technologies. Specifically, attendees will gain an understanding about the features of the F5 steganography algorithm and how the hidden message embedded in a JPEG image by F5 algorithm can be detected.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community, specifically the digital forensics and cybersecurity community, by introducing a possible ML-based approach for detecting steganography generated by using the F5 algorithm. This will help digital forensic as well as the cybersecurity investigators in their case investigations, and the experiences with ML for steganography detection gained in this research will also help researchers in their process of designing and developing the new steganography detection methods.

Steganography is a technology of hiding secret messages by embedding them into the cover media, such as audio, image, video, or text files. Because of its characteristics of detection avoidance and tamper resistance, steganography technology is widely used for secret message communication for both good and bad purposes, and it is increasingly used by cyber criminals for carrying malicious software and launching attacks. Therefore, it is very important for the digital forensics investigators and cybersecurity professionals to have methods to detect the steganography and thwart the cybercrimes.

F5 is a steganographic algorithm of hiding data into JPEG images. It works by changing the Discrete Cosine Transform (DCT) coefficients of a JPEG image. What makes F5 popular for JPEG image steganography is that it is quite strong against both visual and statistical attacks and, therefore, is difficult to detect. Most of the existing F5 steganography detection technologies use the traditional mathematical and statistical approaches to tackle the problem. These technologies possess a higher detection accuracy when the F5 steganography images carry a higher volume of messages. When the hidden message volume is not exceeding the F5 optimum capacity, the accuracy of those technologies is significantly lower.

In the past decade, with the advancement of the computing technology, ML, especially the deep learning technology, has become feasible, and this has opened the door for the numerous applications of these technologies in various fields. With the F5 steganography detection challenge in mind, this research took advantage of the deep learning capability of the Convolutional Neural Network (CNN) to design an ML-based approach to attack this difficult steganography detection problem.

This presentation will provide an overview about steganography, steganalysis, and ML technologies. Focusing on ML for steganography detection, this presentation will discuss the design of the CNN, feature selection and extraction, and training and testing of the developed CNN. The experiences and lessons gained in the design, training, and testing of the CNN for F5 steganography detection will also be discussed.

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### Steganography, Steganalysis, Machine Learning



### C24 Digital Forensic Evidence Cracking an Ingenious Murder Cover-Up: Lochner's Principle Re-Validated

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**Learning Overview:** The goals of this presentation are to emphasize the importance of digital evidence at autopsy, to spread awareness about the mechanisms and technicalities of the mobile phone as an aid to solving crimes, to critically validate Lochner's Principle by the exemplar case, and by elucidating the importance of mobile phone device/Subscriber Identity Module (SIM) cards as useful sources of digital forensic trace evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating that by exploring the exchange of trace evidence, Lochner's principle utilizing non-contact theory may be used as an adjunct to Locard's principle, which propounded on the contact theory. Additionally, the basic working mechanism of digital mobile devices will be introduced.

The increasing usage of mobile cellular devices in daily life, with their inherent functioning technology, has been highly instrumental in death investigations in the recent past. The information stored in mobile devices can ascertain the movement and location of an individual with his contact and communications. There are some unique considerations about mobile phones and associated devices if found as a source of evidence. A major advantage of mobile devices from the forensic perspective is that even deleted information can be recovered from mobile devices in criminal investigations. This is because of the utilization in these devices of flash memory chips, which are physically durable against impact, high temperature, and pressure. Secondly, flash memory has a limited number of writes and can only be erased block-by-block, and mobile devices generally wait until a block is full before erasing data. Furthermore, mobile devices use proprietary wear-leveling algorithms to spread write/erase across flash memory blocks, which can result in deleted data remaining for some time while new data are written to less-used portions of memory. Keeping in mind all the collection, extraction, and analysis-related issues regarding trace evidence, digital evidence serves as excellent sources of information akin in specificity of 100% to fingerprinting when compared to other trace evidence. Nonetheless, the personal nature of these devices makes them easy to establish the last mile evidence required to fix the liability of a device to an individual. There are a growing number of mobile devices for personal organization and communication, many with access to the internet. These devices can be a source of digital evidence in any crime revealing personal information about an individual, including photographs, passwords, and other data. The information they contain can also be instrumental to a crime when they are used to steal intellectual property or create and disseminate child pornography. It has become routine for investigators to collect mobile devices, if available, as evidence in recent years. By far the most obvious trend is that mobile devices will continue to be important in forensic analysis and have large roles in both civil and criminal investigations. Currently, tools and training in this area are limited but, given the rapid increase in their use, this is likely to become one of the largest growth areas in the field of digital evidence examination.

Presented here is an unusual case of the homicide of an unidentified adult female who had been strangled, body tied up, and disposed of by an ingenious means of body sacking. At autopsy, a dead body of an adult female of Asian origin was brought to the mortuary for postmortem examination. One intact red Vodafone SIM card was found perfectly sticking against the back of the body, prevented from falling off or displacement by the brassiere. The cause of death was opined to be asphyxia and venous congestion consequent upon ligature strangulation in a homicidal manner by the ligature material (neck scarf) found *in situ*.

The recovered SIM card was provided to the investigating authorities for conducting prompt investigative operations to nab the suspect. Police tracked the suspect by the International Mobile Subscriber Identity (IMSI) method of phone tapping, thereby directly validating Lochner's principle and proving exclusively important in tracing the accused by temporal, relational, and functional analysis. The recovery of the SIM card played a key role in the identification of the victim, the assailant, and the motive of the homicide.

#### SIM Card, GSM, Digital Evidence



### C25 Quantum Digital Forensics: The Applications of Digital Forensics in a Quantum World

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how quantum computers are poised to impact the future of digital forensic work. Attendees will gain an understanding of how quantum computers function and insight into both the advantages and disadvantages of their application to the digital forensic landscape.

**Impact on the Forensic Science Community:** As with any new technology, digital forensic examiners need to understand how it works and what the impact is to the data they sift through daily to best anticipate both the case applications and the security needs of their industry. This presentation will impact the forensic science community by encouraging discussion on the applications of quantum computers and better preparing the digital forensic community for the anticipated challenges and opportunities of working in a quantum world.

Digital forensic analysts are on the cusp of needing to fight a battle against exponentially more powerful cyber threats. What was once only considered science fiction is now reality, and quantum computers in the wrong hands present a significant problem for those trying to protect individuals and organizations from attackers. The applications for a quantum computer—such as the expansion of cloud computing, a faster internet, unbreakable encryption, and new channels of communication—are also rapidly generating interest in the security industry.<sup>1</sup>

There are significant differences between quantum and classical computers, and some of the problems forensic examiners will face are extracting live data from a system when that data can be in multiple states at the same time, creating exact forensic copies when the state of a bit can change with each observation, capturing data in a transmission when the packet doesn't ever traverse a network, and breaking encryption in a post-quantum environment.<sup>2-4,7,8,10,12-14</sup>

With these challenges also come opportunities. Digital forensic analysts will be able to use quantum encryption to crack old cases that classical computers simply couldn't solve.<sup>5,6</sup> They'll be able to use quantum's pattern-matching technology to scan large databases for matches on faces, locations, and objects of interest.<sup>9</sup> Finally, quantum computers will very likely facilitate the transmission of classical information, making it imperative for examiners to know and understand the architecture they are dealing with.<sup>11,15</sup>

This presentation examines the above questions and discusses the types of forensic needs necessary in a quantum world. This presentation will review how data will be transmitted in a quantum environment, advantages and disadvantages versus classical forensics, the potential for live forensics, pattern recognition for law enforcement applications, and discuss the code-breaking implications for today's current forensic encryption challenges.

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#### Quantum Digital Forensics, Digital Forensics, Quantum Computers



### C26 Applications of a Convolutional Neural Network (CNN) for Automatic Classification of Outsole Features

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**Learning Overview:** After attending this presentation, attendees will be familiar with the ways that CNNs can be applied to classify forensic pattern evidence, specifically with shoe outsole features.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a powerful machine learning method to automatically classify shoe outsoles with the goal of improving the reliability of pattern evidence analysis. Results of this research can be used to automate and speed up shoe print database searches and provide valuable information about the relative frequency of specific characteristics found on outsoles within and across local populations.

The improvement of modern computing has led to the widespread implementation of machine learning, in which computers “learn” about data without being explicitly programmed. CNNs are a form of deep learning that mimics the human brain by using complex networks of hidden layers to learn patterns and make decisions about the contents of an image. For example, we may see an image that contains features such as “eyes” and “nose” and conclude that the image contains a “face.” Similarly, CNNs learn and combine local patterns in a set of training images to learn to detect the presence of a desired set of features in new images.

In forensic applications, images of shoe prints and outsoles are well-suited to classification using CNNs. This is because outsoles often contain distinct and well-defined shapes (e.g., circles, triangles, and text) that can be easily classified by a CNN. Once the shapes contained in a print are detected and classified, the resulting labels can be combined to determine the shoe’s category (e.g., athletic, formal) and brand, and labels for different outsoles can be compared to assess similarity between different designs.

A classification scheme was modified from sets of geometric features currently used by forensic footwear examiners, and thousands of outsole images were obtained and manually labeled according to the new scheme. These images were used to train a new classifier for the convolutional base of VGG16, a pre-trained CNN that is commonly adapted for novel image classification tasks.

One potential application of this new model is to automate and/or speed up database searches that examiners perform when searching for candidate shoe models which may match a questioned print. Another application, which is currently underway, is to collect a large amount of outsole data through sidewalk-embedded scanners, then utilize statistical methods to determine the relative frequency with which certain characteristics appear in shoes that are commonly worn within specific populations.

This presentation will include discussion of the process and challenges of developing a classification scheme and training a CNN for outsole recognition, summarize the performance of the current model, and present preliminary results for the determination of feature frequency in local populations.

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#### Machine Learning, Shoe Outsole, Pattern Evidence





### C27 An Effective Security Assessment Framework for Drone as a Service (DaaS): A Digital Forensic Technique

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**Learning Overview:** After attending this presentation, attendees will be able to clarify security risks of drones, determine the best practices of security assessment, and make decisions related to the security of drones during operation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring the best practice techniques related to drone penetration testing, including an effective methodology to keep continuity of risk-free drone operation, especially drones used for emergency purposes, such as safety and rescue.

This presentation will raise awareness in the forensic science community as it draws the importance of securely operated drones for first responders. This presentation will cover various aspects of security, including data transmission, software restrictions, and embedded system-related events. In order to propose a security assessment for drones, they incorporate digital forensics and penetration testing techniques to suggest secure methods related to drone operations. Therefore, this research enhances the security level of flying devices and the overall digital forensics procedure in case of a disruption incident.

Firmware analysis and penetration testing on embedded devices is crucial today. Firmware is used in most emerging technologies, which make them an important factor in securing these embedded devices, such as drones. This presentation will cover aspects of security assessment conducted on three different types of drones used by safety and rescue organizations. The presenters focus on the analysis of firmware to determine both security vulnerabilities that could expose threats to the activity of drones, software restrictions that could limit the operation of drones, such as No Fly Zone (NFA), and operational communication commands. The use of DaaS is rapidly increasing, and risk assessment of software-related issues is important to support the adoption of DaaS in a secure operational manner.

The analysis in this work will be performed based on well-known security measures, such as the Open Web Application Security Project (OWASP) Internet of Things (IoT) top 10, the National Fire Protection Association (NFPA) 2400, standard for Small Unmanned Aircraft Systems (sUAS) used for public safety operations, and the National Institute of Standards and Technology (NIST) security guidelines, and develop a recommendation on the attack resistance based on conducted security auditing of the firmware.<sup>1</sup> Finally, this work will be conducted on DJI Matric, DJI Mavic, and Parrot Bebop, which are currently used by most of the safety and rescue organizations.

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**DaaS, Penetration Testing, Firmware Analysis**



### C28 The Forensic Utility of Smart Doorbells

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the current forensic utility of smart doorbells in regard to data extraction and image quality.

**Impact on the Forensic Science Community:** This presentation, a research project conducted by the Office of the Chief Scientist (the Research, Development, Testing, and Evaluation arm of the Defense Forensic Science Center), will impact the forensic science community by demonstrating how and what types of data may be extracted from smart doorbells, as well as demonstrating if the image quality is sufficient for image analysis.

There are numerous SmartHome devices currently available to the consumer, from home automation hubs to function-specific items. Valuable data can be recovered from such devices that may be useful to forensic investigations. Smart doorbells are devices with cameras, microphones, speakers, and motion-sensing abilities that operate via a mobile device or through Wi-Fi. Through their normal use, smart doorbells may capture visual information about a suspect, timeline, or series of actions during, before, and/or after the commission of a crime. Such information could impact the investigation of a crime where breaking and entering through a doorway occurred.

With the increased use of smart video doorbells, there is a need to determine appropriate data extraction methods, the extent to which data can be recovered, and the image quality of the recovered data. The current research examines two smart doorbells with video-recording capabilities to examine their potential for future forensic applications. Both an Amazon® Ring® and Google® Nest Hello™ video doorbells were used in the course of this research. These two devices were chosen based on availability to the researchers. Both devices offer live video-streaming and motion-sensing abilities.

Each device was installed on the doorframe at a traditional doorbell height. Placement was viewed to ensure the area being captured would properly capture people in proximity to the door. Researchers used each device over the course of several months in order to collect recordings from various times of the day and weather conditions. During this time, each doorbell was in continuous use; however, recording only took place when there was motion to activate the doorbell. At the completion of this timeframe, an extraction was conducted on each mobile device used to access the doorbells (iPhone® 7 Plus and iPhone® 6) using standard computer forensic methods. The two methods of extraction employed a Physical Analyzer. First, a physical extraction was performed. Second, a logical extraction was completed. Results of the extractions were compared to determine what type of data was retrievable and what affects the extraction had, if any, on image quality. The image quality was further assessed through an analysis of the metadata (e.g., resolution and file size) and visual analysis of the details present (e.g., scars, facial features, hats, and shirt tags).

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#### SmartHome, Doorbells, Image Analysis



### C29 Counterfeit Drug Detection Using Multi-Spectral Imaging

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**Learning Overview:** The goal of this presentation is to: (1) provide instruction on multi-spectral imaging theory and measurement; (2) describe how multi-spectral imaging can be used to detect counterfeit drug products and packaging; and (3) provide case study examples of counterfeit drug detection using the Variable Magnification Lens (VML).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing multi-spectral imaging as an effective tool to help forensic teams identify counterfeit products or packaging in a fast, non-destructive manner.

Counterfeit pharmaceutical products are an increasing threat to patients and pose severe challenges to the pharmaceutical industry. The challenge for forensics teams searching for counterfeits is that the counterfeiters are becoming more sophisticated, making it increasingly difficult to distinguish authentic products from replicas. These teams need superior detection techniques. Multi-spectral imaging has been utilized in the field of satellite and airborne sensing for many years but is now available in a powerful benchtop laboratory analyzer. The system consists of a high-resolution camera that takes a series of images at wavelength bands ranging from ultraviolet to near-infrared light. Because a unique image is taken at each wavelength band, the images can be viewed individually or in combination. In addition, specific layers within the image can be isolated and compared. Multivariate analysis can then be utilized to identify differences between the spectra of each layer over the entire wavelength range.

The objective of this study was to utilize multi-spectral imaging to identify counterfeit pharmaceutical products. Three different tablet types consisting of both genuine and counterfeit samples were analyzed using multi-spectral imaging. A color image of the products did not reveal any differences, nor were differences detectable by the naked eye. However, by observing the entire spectra, it became clear that while distinguishable differences did not exist in the visual band, differences could be observed in the near-infrared region. Authentic and counterfeit samples were then compared using normalized Canonical Discriminant Analysis (nCDA) to identify the best way to differentiate between the samples. Once trained by nCDA with the correct combination of bands to use, the system was able to provide a spectral fingerprint to correctly identify genuine product from counterfeit in blind samples. Multi-spectral imaging was able to identify counterfeit tablets in blind samples with 100% accuracy and could even detect counterfeit tablets while still in the package, eliminating the need to remove the tablets for analysis. In addition, multi-spectral imaging was able to distinguish counterfeit packaging from authentic with full accuracy. Multi-spectral imaging was concluded to be an effective tool for forensics teams to identify counterfeit products in a fast, non-destructive, and versatile manner.

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**Counterfeit, Imaging, Fast**



### C30 Pathways to the Identification of Multinational Victims of Mass Disasters: The Role of Blockchain Technology

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**Learning Overview:** After attending this presentation, attendees will have learned, using real-world cases, how blockchain technology adoption can achieve an automated archiving system to fully manage the identification of medical and dental data for comparison and data mining that should promote novel solutions for forensic human identification and Disaster Victim Identification (DVI).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how blockchain technology adoption should revolutionize the human identification and disaster management processes worldwide, improving the management of missing persons, Antemortem (AM) data repositories of living people, Postmortem (PM) data repositories of recovered unidentified human remains, interactions with electronic medical and dental records, and helping to facilitate the comparison of compatible biological profiles for definitive identification. The possible applications presented in this study promise enormous benefits to authorities, victims, their families, and society in general. This presentation should create a trusted ecosystem to help forensic science practitioners advance beyond traditional methods and stimulate innovative collaborations with computer scientists in order to improve procedures, especially in the area of forensic dental identification.

In mass disasters with multinational victims, it is critical to identify the deceased for judicial, ethical, religious, and human rights reasons, as well as to allow the next of kin to complete the grieving process. The DVI process is a complex procedure in which PM identifying data, essentially fingerprints, DNA, and dental, is collected, then compared with equivalent AM data related to the missing person's list. Although there are solutions used in the field of human identification, they all fall short of equipping practitioners with the tools needed for achieving human identification in a timely manner. Initially, it is significantly challenging to manage missing person's lists containing years, and sometimes decades, of family AM data resources' updates. Furthermore, there is currently no record of any holistic and decentralized technical solutions for managing both AM and PM data for human identification to support collaborative multinational and interjurisdictional processes. Blockchain technology provides the tools to facilitate building trustworthy, secure, and holistic ecosystems, and it can disseminate siloed AM and PM data across systems, protecting data breaches, redundancies, inconsistencies, and errors. As such, blockchain technology can revolutionize the human identification process worldwide in terms of managing missing person's lists, AM data repositories for living people, PM data repositories of recovered unidentified victims, and contribute to the comparison of compatible biological profiles for definitive identification.

This study presents real-world use cases that examine how this technology facilitates multi-jurisdictional data information-sharing in conjunction with the forthcoming circulation of patients' electronic medical and dental records. This study encourages openness and interjurisdictional victim information-sharing to paint a full picture that would allow each team to perform their tasks. However, this raises information security and privacy concerns that could hinder its adoption by some DVI teams that have to comply with tighter jurisdiction-specific data protection laws and regulations that are not applicable elsewhere, such as the General Data Protection Regulation (GDPR), considered the toughest privacy and security law applied today.

#### **Blockchain Technology, Disaster Victim Identification, Forensic Odontology**



### C31 A Proposed Framework for Digital Video Authentication

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**Learning Overview:** After attending this presentation, attendees will understand the proposed authentication process for digital video using methods based upon scientific research and publication in which various analyses may be combined into a framework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a logical and scientifically sound process for digital video authentication.

Digital video authentication is a complex process of establishing the provenance of a questioned recording to determine whether it is consistent with an original recording or if there is evidence of alteration. One simply has to look at news outlets or social media to see that our society video records events from births to deaths and everything in between. A trial court's acceptance of videos supporting administrative hearings, civil litigation, and criminal cases is based on a foundation that the videos offered into evidence are authentic; however, technological advancements in video editing capabilities provide an easy method to edit digital videos.

The proposed framework offers a structured approach to evaluate and incorporate methods, existing and new, that come from scientific research and publication. The proposed framework incorporates methods or techniques that are evaluated for reproducibility, repeatability, accuracy, and precision while meeting the general legal requirements (a general application of the *Daubert* standard) recognized by courts in the international community, United States, and many countries around the world.

This presentation offers a quick overview of digital video file creation chain. This also includes factors that influence the final digital media streams and a general description of camera sensor noises for both Complementary Metal-Oxide-Semiconductor, and Charge Couple Device type sensors.

This presentation addresses the overall development and proposed use of the framework, previous research of analysis methods/techniques, testing of the methods/techniques, and an overview of the testing results. The framework provides the forensic video examiner a structured approach to subjecting the questioned video file to a series of smaller tests while using previously published and forensic community-recognized methods/techniques. The proposed framework also has a proposed workflow optimization option for use by management in an effort to manage resources and personnel. The framework has a built-in methodology evaluation tool based upon a general application of the *Daubert* standard. The methodology evaluation tool includes a methodology validation assessment and a legal assessment to aid the forensic video examiner in determining if a proposed method should be included or excluded from use as part of the specific framework protocol for each video file considered for authentication. Also, the proposed framework offers the forensic video examiner a methodology to assess published video and audio authentication techniques recognized in the forensic science community while using generally accepted criteria to test and evaluate the techniques as expected by the courts.

This presentation will also note some limitations to the use of the proposed framework for digital video authentication. The limitations include the following: acceptance of the proposed framework for video authentication by the courts will always be based upon a case-by-case basis dependent upon each case's facts, proper use of the scientific methods, and the overall experience, training, and knowledge of the forensic video examiner who testifies as an expert; the proposed framework is intended for digital video and is not applicable to analog video; and no scientific inquiry, including those in media forensics, produce results of absolute certainty—this includes digital video authentication. The proposed framework uses a philosophy that avoids implying 100% certainty with the exception of known alterations or deletions applied in the underlying testing/experiments for the development of the proposed framework.

This presentation will also note that the framework is designed for incorporation of new digital video authentication methods that are developed in the forensic science community, as they are proven to be based upon scientific research and publication and are accepted in the forensic science community.

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#### Digital Video, Authentication, Camera Sensor Noise



### C32 Automated Standards-Based Normalization and Correlation of Mobile Device Evidence

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**Learning Overview:** This presentation will provide attendees with a more efficient, reliable, standards-based approach to automatically normalize, combine, and correlate digital and multimedia evidence. This presentation provides a fit-for-purpose solution using the evolving Cyber-investigation Analysis Standard Expression (CASE) standard to represent information extracted using mobile device forensic tools and to combine this information into correlation analysis tools. This work concentrates on treating the following categories of information: (1) communications (Short Message Service [SMS], chat messages, email); (2) identifiers (telephone numbers, email addresses, social media accounts, Internet Provider [IP] addresses); (3) temporal indicators (timestamps, call durations); and (4) spatial indicators (Global Positioning System [GPS] coordinates, addresses).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) saving time correlating and analyzing digital/multimedia evidence from mobile devices; (2) reducing risk of errors and omissions combining and correlating digital/multimedia evidence from mobile devices; (3) increasing completeness of forensic analysis of digital/multimedia evidence; and (4) tracking chain of evidence throughout the export and combination process to allow forensic analysts to track findings back to their origin.

Increasingly, mobile devices contain large amounts of digital evidence relevant to criminal investigations.<sup>1</sup> This digital evidence can be analyzed to make inferences about identities, locations, chronologies, and relationships between relevant entities to address critical questions in criminal investigations.<sup>2</sup> However, there are severe limitations in current capabilities to combine and correlate all available digital evidence. First, no single mobile device forensic tool can extract all types of digital evidence. Second, tools export data in different formats, without consideration for interoperability. Third, it is necessary to combine the results from multiple tools to obtain comprehensive visibility across all digital evidence. Fourth, mobile device forensic tools do not maintain chain of evidence throughout the export and combination process, making it difficult to track forensic findings back to their source. Dealing with these problems, forensic practitioners reformat and combine information from different sources by hand, which is a laborious and time-consuming process that can result in errors and omissions.<sup>3</sup> For example, using spreadsheet software or database applications to import and format data from various sources can result in items such as date-time stamps being altered, entries not being imported, and other problems that negatively impact forensic analysis. There is a need for automated combination and correlation between datasets processed by mobile device forensic tools.<sup>4</sup>

The specific educational objectives of this presentation are to: (1) raise attendee awareness of current limitations in mobile forensic tools and how to address these limitations; (2) teach the attendees how digital/multimedia evidence extracted using mobile forensic tools is automatically translated into the CASE standard; (3) inform attendees about open source resources for implementing CASE in existing tools and systems; (4) demonstrate the value of importing normalized digital/multimedia evidence from mobile forensic tools into a platform for correlation and analysis; and (5) provide a roadmap of work to strengthen and expand CASE adoption across the digital forensic community.

This work addresses challenges in the way different tools represent extracted data, including missing information, only displaying one party in communications, and variations in the format of common information (e.g., timestamps, phone numbers).

By implementing the CASE standard, this solution does not require tool developers to alter their data model. Instead, it is necessary to translate their data model into a community-developed ontology for representing cyber-investigation information. This approach allows visualization tools, such as Cellebrite® and Analyst's Notebook®, to automatically (rapidly) import data from all sources into a cohesive and comprehensive picture to support selection, correlation, and analysis.

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3. Casey, Eoghan; Barnum, Sean; Griffith, Ryan; Snyder, Jon; van Beek, Harm; Nelson, Alex. 2017. Advancing coordinated cyber-investigations and tool interoperability using a community developed specification language. *Digital Investigation*, Issue 22.
4. Reif, Martina. 2019. *L'implémentation de CASE aux extractions téléphoniques*. Master's Project, University of Lausanne.

#### Digital/Multimedia Evidence, Standards-Based Correlation, Chain of Evidence



### C33 Known Source Artifacts Examination With Digital Forensic Tools

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**Learning Overview:** After attending this presentation, attendees will understand the capability of various digital forensic tools when used to examine devices with known, documented activities. Recovery of artifacts related to activity is attempted by using each tool. Each tool's data extraction will be reviewed for the tool's ability and accuracy of locating the artifact.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing knowledge regarding how forensic tools extract known expected artifacts. This knowledge will help examiners validate examination results and know which tool could be used to get a more precise representation of what has occurred on a device. This presentation will not only give examiners knowledge on forensic tools for Windows® computers, but also for mobile devices.

In the field of digital forensics, forensic software plays a major role in obtaining the evidence used in convicting or excluding someone of a crime. Multiple different digital forensic tools have come to the market in recent years and all these tools are claimed to be the best for all the examiner's needs for extracting and reporting digital evidence. However, because these tools process the data using different methods and present the recovered evidence in various ways, it is very challenging for the examiners to know how each tool behaves. In order to overcome this challenge, it is important for digital forensic investigators to have the knowledge of what tools are best at doing certain tasks they want the tools to do. This presentation is designed to provide digital forensic practitioners with that knowledge.

Digital forensic examiners must prepare every examination as if it were to be presented in court. Having confidence in the tools and methods used for data extraction is a critical element for testimony. This presentation will provide attendees with information about effectively comparing the forensic tools and how the results can vary depending on the software used. Windows® computers and mobile device technology change at a rapid rate and the amount of people who use mobile devices grows every year. With more devices that are constantly changing, it has become increasingly important that examiners be aware of the differences in examination results and know how to validate their findings.

This presentation will provide an overview of methods to validate different forensic software tools and their capabilities for discovering related digital evidence on Windows® computers and mobile devices.

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### Digital Forensics, Artifacts, Digital Forensic Tools



### C34 Teaching Digital Forensics to Young Women and Underserved Youth

*Eoghan Casey, PhD\*, University of Lausanne, Lausanne, Vaud 1015, SWITZERLAND; Daryl Pfeif, BA, Digital Forensics Solutions, New Orleans, LA 70130*

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**Learning Overview:** After attending this presentation, attendees will better understand: (1) the overarching design principles and research underpinning this innovative educational initiative; (2) practical lessons learned throughout the development and implementation of the Digital Forensic Science Learning Environment (DFSLE) and related activities; and (3) educational and infrastructure challenges and resulting solutions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) bringing more women and underserved populations into traditionally underrepresented computer science-related fields; (2) enhancing Science, Technology, Engineering, and Math plus Computing (STEM+C) education at out-of-school settings; (3) increasing the number of qualified candidates for a rapidly growing number of digital forensic science positions; (4) providing a robust instructional framework for teaching science inquiry-based subjects; and (5) promoting responsible online behavior and cyber street smarts.

Attendees will learn about an initiative to address the growing demand for qualified candidates in digital forensics, particularly young women and men from underrepresented populations. This presentation explains the design, development, implementation, and future of the DFSLE, an innovative educational program and supporting virtual learning laboratory created to provide youth with digital forensic knowledge, skills, and career pathways. This program combines online and in-person classroom elements that challenge students to become cyber sleuths solving real-world problems, applying methods and tools in digital forensics, and exploring complex social and technical issues associated with cybercrime. The DFSLE extends the Investigate and Decide Learning Environment (IDLE) framework from learning sciences, teaching technical and problem-solving skills through goal-based investigative scenarios. By immersing students in real-world problem-solving situations, this initiative strives to motivate students to develop scientific reasoning, technical knowledge, practical skills, and pursue related careers while improving their cyber street smarts. This initiative has included students from grades 9-12 in Baltimore, MD, New Orleans, LA, and Seattle, WA. The project connects with aspects of expectancy-value theory and Social Cognitive Career Theory (SCCT) to encourage students to pursue educational or career pathways related to computer science and STEM. In particular, this focuses on the community and relationship-building elements of the mentor network and collaborative peer-learning activities to provide strong social and environmental supports. The practical curriculum has been augmented with teacher training, classroom dashboard, lesson plans, and a pathway resource guide. Classroom activities introduce students to experts in the domain as role models and career mapping. In this manner, this initiative guides students along pathways that can lead to community college and university degree programs and, ultimately, into the workforce.

**Sustainability:** The Computer Science Study Lab (CSSL) is being promoted in partnership with the National Girls Collaborative. Further work on teaching training and open source training datasets is underway to ensure long-term sustainability of this initiative.

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**Digital/Multimedia Evidence, Forensic Science Education, Computer-Assisted Training**





### C35 iOS® Photo Vault Forensics

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**Learning Overview:** After attending this presentation, attendees will be aware of what artifacts may be forensically recovered from photo vault applications using iOS®.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing relevant information for digital forensic investigators who may need to identify and recover relevant artifacts from a vault application on the iOS®.

Modern digital devices, such as laptops, personal computers, mobile phones, and tablets, have vault apps—applications that hide photos, videos, and texts in a secure “vault.”<sup>1</sup> These vault applications allow the user to securely store their personal data, which makes it difficult for anyone except the device’s owner to view the files, even if they have access to the device.<sup>2</sup> These mobile vault applications often disguise themselves by pretending to look like other applications, such as a calculator, or only display information when the user enters a valid password.

From a law enforcement investigation perspective, offenders may use vault apps to hide illegal images, such as child sexual exploitation materials or illicit text messages with minors. In cases such as these, vault applications may serve as a hindrance to law enforcement. While traditional digital forensic tools may be able to recover photos directly stored on the phone, they may not be able to find those secured by photo vaults.<sup>3</sup>

While studies have examined vault applications on Android® operating systems, limited research exists using the iPhone® or iOS® ecosystem. The current study had four aims: determine what information can be forensically recovered from vault applications, examine implications for user privacy, document the methods for forensically extracting information from vault apps, and compare the results from different digital forensic tools. The five most popular vault applications in 2019 on the iOS® store were analyzed. Various types of photos were uploaded to the vault applications using the following techniques: creating a photo by taking a screenshot, saving a photo from a text message, saving a photo from a browser, and using the phone’s camera to create the photo. This study also compared the results of various digital forensic tools (e.g., Cellebrite®, Axiom®). The results will be fully discussed and recommendations for the digital forensic examination of photo vault apps on the iOS® ecosystem will be provided.

#### Reference(s):

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#### Photo Vault, Digital Forensics, Mobile Forensics



### C36 A Constantly Moving Target: Best Practices for Apple® iOS® Device Seizure, Access, and Extraction

*Joseph Levi White, MS\*, Defense Forensic Science Center, Forest Park, GA 30297*

**Learning Overview:** After attending this presentation, attendees will have an understanding of the current best practices for seizing Apple® iOS® devices to ensure a higher probability of obtaining access to any valuable maintained data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the current best practices for seizing and processing various Apple® iOS® mobile devices. This presentation will demonstrate how the methods used during seizure and submission to a laboratory may dictate the potential unlocking capabilities, extraction capabilities, and types of data that can be obtained.

Evidence maintained on mobile devices, such as the Apple® iPhone®, may be critical to a case, but the advanced security measures (encryption, Personal Identification Number (PIN)/passcode lock, pattern lock, fingerprint, facial recognition, etc.) enabled on the majority of newer mobile devices to protect the owner from unauthorized access to their personal device also stands in the way of the digital forensic examiner. The actions taken by the first responder, the initial person to seize and/or interact with the mobile device, the evidence custodian, any triage personnel, etc., all may have a drastic impact on what can be done to obtain data from that device later in a laboratory setting.

Many, if not most/all, law enforcement/crime scene agencies and forensic science laboratories adhere to strict methods and/or policies and procedures for seizing evidence, conducting an inventory, photographing the evidence, processing the evidence through the laboratory, and issuing reports. These policies and procedures are required for obtaining and maintaining agency/laboratory accreditation. The problem lies within the fact that many of these policies and procedures, if adhered to, will result in either the loss of digital data or the loss of access to digital data maintained on locked iOS® devices.

This presentation will highlight multiple current typical mobile device seizure/processing methodologies and their potential impact on casework. As specific actions are taken with an iOS® device, access and security features utilized by laboratory personnel to gain access to the device may be altered. Something as simple as removing the Subscriber Identity Module (SIM) card from the device for evidence inventory can disable future biometric (facial identification or fingerprint) access to the device. Powering off mobile devices at seizure is a typical practice for evidence storage and transfer, but this action changes the lock state on iOS® devices from After First Unlock (AFU) to Before First Unlock (BFU) mode. This change from one of the device's least secure states (AFU) to one of the most protected states (BFU) causes an iOS® device to block access to various types of data maintained on the device. The timeline from device seizure to obtaining access to maintained data has become very critical. With each passing second, there is a greater potential for evidentiary data to be deleted from the device or blocked from future access.

Due to seemingly constant security and feature updates to the Apple® iOS® operating system, best practices for iOS® device seizure and processing are constantly evolving. With each new software release, digital forensic examiners must be willing to adjust and implement changes to methods and/or policies and procedures quickly to remain steady on the moving target of defeating advanced mobile device security.

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**Apple® iPhone®, Mobile Forensics, Cell Phone Forensics**



### C37 Unlocking Fingerprint Scanner-Enabled Mobile Phones

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the efficiency and effectiveness of using recorded fingerprints to unlock fingerprint scanner-enabled mobile phones.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the benefits and limitations of a novel technique of using recorded fingerprints to unlock fingerprint scanner-enabled mobile phones.

This presentation, a research project conducted by the Office of the Chief Scientist (the Research, Development, Testing, and Evaluation arm of the Defense Forensic Science Center [DFSC]), focuses on the benefits and limitations of a novel technique of using recorded fingerprints to unlock fingerprint-scanner-enabled mobile phones.

Mobile devices are frequently submitted as evidence in digital forensic examinations. While law enforcement officials are required to secure a search warrant prior to the examination of mobile devices, there are often issues accessing locked phones even after a search warrant is obtained. While suspects may willingly provide a passcode to a locked mobile phone, there are also numerous occasions when a passcode is not provided (e.g., death, uncooperative suspect or victim, etc.). A suspect cannot be required to provide a passcode; however, to conduct a thorough digital forensic examination, it is imperative that examiners are able to access the mobile device's data in a timely fashion. While there are technologies that may assist an examiner in cracking the passcode, often such solutions are expensive and time-intensive. Furthermore, security features of mobile phones may erase data after failed passcode unlock attempts, so it is essential that an alternate method of unlocking a mobile phone is available.

Recently, several articles have demonstrated the possibility of unlocking fingerprint scanner-enabled mobile phones using printed images of fingerprints. A process using a standard inkjet printer combined with silver, conductive inks, and specialty paper can be used to generate a fingerprint capable of unlocking a mobile phone. While a technique has been roughly outlined and has been demonstrated, the success of this process requires further investigation in order to be standardized and employed with commercially available products for practical applications in a crime laboratory setting.

While the articles cite specific instances where mobile phones have been unlocked, recorded prints have not been used as a part of this process. Furthermore, the mobile phone itself may be host to a latent fingerprint, which could be imaged and used to unlock the phone. Within a criminal investigation laboratory, both of these sources of fingerprints have potential for assisting in a digital forensic examination.

Three fingerprint scanner-enabled mobile phones (Samsung™ S6 Edge+, Samsung™ Galaxy S7, and iPhone® 5S) were selected, based on their availability within the Documents & Digital Evidence Branch at the DFSC. A total of six subjects provided record prints (inked and LiveScan), and each participant enrolled his or her right and left thumb and index fingers in each of the mobile phones. Enrolled fingerprints were removed from each phone between participants. Three male and three female subjects were used to produce a sample set of fingerprints that varied in subtle differences in the ridges (e.g., thickness, widths, etc.). The variation among the donors was used to demonstrate the feasibility of the approach across multiple individuals in the general population.

The recorded prints were scanned and enhanced. An inkjet printer with silver, conductive inks was used to print the fingerprints on the appropriate specialty paper. Each printed fingerprint was used to attempt to unlock the mobile phones using various methods. Multiple image enhancements were also made to test the efficacy of the printed fingerprints. Each print was tested multiple times in a systematic manner to determine the repeatability of the technique. The results of each process were recorded and compared to illustrate the differences between participants, mobile devices, image enhancements, and application of the fingerprint to the scanner (heat and/or pressure).

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### Unlocking, Mobile Phone, Fingerprint Scanner



### C38 A Forensic Comparative Analysis of a Fitness Tracking Application on Mobile Devices Assigned

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the current capabilities of extracting the mobile app, STRAVA™, Global Positioning System (GPS) data from mobile devices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the benefits and limitations of the extraction techniques used for obtaining GPS data that is recorded through the STRAVA™ app.

This presentation, a research project conducted by the Office of the Chief Scientist (the Research, Development, Testing, and Evaluation arm of the Defense Forensic Science Center), focuses on the benefits and limitations of the extraction techniques used for obtaining GPS data that is recorded through the discussed mobile fitness-tracking app.

Digital Forensic Examiners (DFEs) are responsible for extracting data from various electronic devices and performing analyses on different data types. It is the responsibility of the Digital and Multimedia Evidence (DME) community, including DFEs and researchers, to determine how to extract and interpret the data as well as discover if the data may be a potentially useful source of information. With the expansion of body-worn, fitness-tracker devices, the DME community searches for potentially pertinent data stored within fitness-tracking devices and the companion client devices that store the data.

The example body-worn, fitness-tracking app is used to track athletic activity via satellite navigation. It also works with GPS-enabled watches and head units. Tracking the user's activity may be forensically useful in establishing a suspect's location on a specific date and time. As such, understanding the data and establishing an extraction procedure will enable DFEs to successfully extract physical activity pattern data in cases where a suspect's or victim's location or activity may be important to establish.

Two companion client devices (iPhone® 7 Plus and iPhone® 6) were used for this research based on their availability to the researchers. The premium version of the mobile fitness-tracking app was loaded to the iPhone® 7 Plus. The basic version was loaded to the iPhone® 6. The companion clients were used to record a number of activities (e.g., running, walking, cycling, etc.) to generate data. Generated data included distances, locations, times, and dates to show each user's activity pattern. Recording only occurred when the app was activated. A manual log of each user's activities was kept for post-extraction data comparison. Data was collected over several months to establish the user's activity patterns and to collect various fitness activities. After several months, physical (full, including deleted data) and logical (partial) extractions were conducted on each device using standard computer forensic methods. Results were analyzed for accuracy by comparing the app's data to the user's manual log, as well as comparing the data recovered through the different extraction techniques. Through the evaluation of the app and the data extracted, investigators and examiners will be provided with relevant information on the steps to take when recovering and examining mobile devices and the GPS data that could be associated with a crime.

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#### Mobile Device Forensics, Cell Phone Forensics, Digital Extraction



### C39 Android™ App Forensic Evidence Database (AndroidAED)

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**Learning Overview:** After attending this presentation, attendees will better understand how AndroidAED will be beneficial for academic researchers whose studies relate to mobile applications that grant them the ability to search through many of the available applications across various third-party app stores.

**Impact on the Forensic Science Community:** AndroidAED, per this study's research, is the first Android™ app forensic evidence database with the highest precision and the most comprehensive coverage in discovering evidence generated from Android™ apps. This presentation will impact the forensic science community by illustrating how digital forensic investigators can significantly improve the process of investigation of evidence from mobile devices.

With more than 2.7 billion smart phone users across the world, it is no surprise that the mobile app industry is thriving.<sup>1</sup> Android's™ smart phone share increased to 86.7% in 2019 and has remained at more than 75% in the past ten years. A recent study of globally available apps by AndroZoo shows that the number of apps has exceeded eight million and is still rapidly growing.<sup>2</sup> Current digital forensic practices for finding data on these apps is limited, time consuming, and error prone. As an example, Cellebrite® UFED, a commercial mobile device forensic tool, only supports the profiles of 6,000 apps.<sup>3</sup> If mobile devices contain apps that are not included in the 6,000 apps, it cannot work properly. Therefore, an up-to-date, real-world evidence database of Android™ apps from multiple app stores—AndroidAED—is being built employing two analyzing tools developed by this study, namely static and dynamic EviHunter, respectively.<sup>5,6</sup> Web crawlers are created to traverse app stores to collect metadata and download application files. Since each site has a different design and functions, a crawler tailored to each store to collect data was created. The crawlers utilize BeautifulSoup to scrape each webpage and the Selenium driver for webpage interactions. Currently, seven crawlers have been completed and 40 more are under development. The currently supported app markets include: Google® Play Store, APKPure, Uptodown, APKMirror, Aptoide, and F-Droid.<sup>4,7-11</sup> After collecting the file, it will be processed using the forensic analysis tools, collecting where the application is storing the evidentiary information. The information about the application that is collected from each store webpage gets stored into a MongoDB instance. The actual APK files of the applications are stored in a separate file system, and the path to the file is linked to the app entry in MongoDB. In this way, digital forensic investigators can simply query the database to find all the possible evidence data (e.g., locations, photos, call logs, time, etc.) generated by the app and corresponding evidentiary file path. Moreover, considering that the apps installed on the suspect's device can vary on the app's version and source of installation (app store), AndroidAED hosts all the available versions of apps that have been collected from various app markets.

The main contributions of AndroidAED are summarized as follows: (1) AndroidAED, per research, is the first Android™ app forensic evidence database with the highest precision and the most comprehensive coverage in discovering evidence generated from Android™ apps; (2) AndroidAED will be accessible for digital forensic investigators and significantly improves the investigation of evidence from mobile devices; (3) AndroidAED will be available for academic researchers whose studies relate to mobile applications that grant them the ability to search through many of the available applications across various third-party app stores; and (4) AndroidAED will keep updating to provide the most up-to-date evidentiary data for real-world apps, ranging from very popular to very unpopular, available from app stores around the world.

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#### Android™, Database, Evidence



### C40 The Use of the On-Screen Time Display for Authentication of Body-Worn Video

*Gretchel Lomboy, MSc\*, Seattle, WA 98115; James Zjalic, MSc, Verden Forensics, Edgbaston, England B15 1TH, UNITED KINGDOM*

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**Learning Overview:** After attending this presentation, attendees will better understand the need for the development of methods for the authentication of Body-Worn Camera (BWC) recordings and will be presented with the results from research performed in relation to such.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing one method of authenticating body-worn video in a matter that can be utilized in casework with immediate effect.

The proposed method takes advantage of the on-screen time display of BWC video recordings, which is superimposed onto video frames during encoding. It is based on the assumption that as this is added during the encoding stage, factors relating to the capture environment, such as lighting, time of day, camera movement, and recording length, have no influence on parameters such as the pixel values used to represent it and the resolution of both its digits and the display in its entirety. It, therefore, stands to reason that its representation should be consistent across recordings captured with a device of the same make and model. As transcoding decodes and re-encodes the visual content in a different format, it can also be inferred that this embedded trace will be changed when a conversion occurs, as would be the case when editing and uploading to social media.

The increase in the use of BWCs by law enforcement, combined with the recent trend for videos from such to be shared on both social media and by news and media outlets, has made the authentication of these recordings of more importance than ever before.

To address the above, the following hypotheses were proposed, in sequential order: (1) the on-screen time display within BWC video streams from a specific make and model of device is consistent regardless of all other factors; (2) the on-screen time display within BWC video streams changes when the video is transcoded from the original recording; therefore (3) the on-screen time display within BWC video streams can be used to determine the authenticity of a recording.

To test the proposed hypotheses, a dataset of original recordings was developed through the capture of videos in different environmental conditions, under differing light sources, and of varying lengths. Various parameters of the onscreen time display related to resolution and pixel values were then measured to allow the intra-variability (between captures from the same device) and inter-variability (between captures of differing devices) to be determined. The video data was then transcoded to new formats and comparisons made between the measurements from parameters of the original recordings and their transcoded versions was performed.

This research aims to provide a stepping stone for further developments in methods of authentication of body-worn video.

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#### **Body-Worn, Video, Authentication**



### C41 Narrative Use Cases for Harmonizing Forensic Science Practices and Digital/Multimedia Evidence

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**Learning Overview:** Attendees of this presentation will learn to apply core forensic science processes to cases involving digital/multimedia evidence. After attending this presentation, attendees will have a clearer understanding of how to integrate digital/multimedia evidence and forensic science by: (1) learning how to apply core forensic concepts and principles (as defined in the Organization of Scientific Area Committees [OSAC] technical publication 002) to digital/multimedia evidence; (2) comprehending the use of narrative to understand and explain forensic treatment and interpretation of digital/multimedia evidence; (3) emphasizing the importance of narrative in forensic science; and (4) raising the awareness of judges, attorneys, and juries of the uses and value of digital/multimedia evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) strengthening scientific foundations of digital/multimedia evidence; (2) increasing the practical application of core forensic concepts in digital/multimedia evidence; (3) clarifying the importance of narrative in conveying forensic science concepts and results; (4) increasing participation in generating and refining use cases for digital/multimedia evidence in forensic science; and (5) raising awareness of judges, attorneys, and juries as to the uses and value of digital/multimedia evidence.

There is a need for increased integration of digital/multimedia evidence into forensic science.<sup>1</sup> At the same time, there is a need for digital transformations in forensic science.<sup>2</sup> The National Institute of Standards and Technology Organization of Scientific Area Committees (NIST OSAC) process has encouraged all forensic science disciplines to explore their foundations and practices; it has also revealed the commonalities between them.<sup>3</sup>

Narrative theory plays an important role in forensic science in clearly conveying concepts and findings using a framework and context that people can easily comprehend.<sup>4</sup> Taking this approach, this presentation will guide forensic practitioners through the practice of applying core forensic processes to actual digital forensic investigations. Specifically, this presentation introduces a project out of the NIST OSAC that is creating a reference database of narrative use cases involving digital/multimedia evidence that capture the methods and outcomes in relation to underlying core forensic questions and processes. Narrative use cases in this reference database cover forensic analysis of digital/multimedia evidence, including speaker comparison and facial comparison.

This work is part of the OSAC Digital/Multimedia Scientific Area Committee (SAC).

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#### Digital Transformations, Narrative Use Cases, NIST OSAC



### C42 Digital Evidence in the United States Courts of Appeal

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**Learning Overview:** After attending this presentation, attendees will understand: (1) the most common legal basis for appeals related to digital evidence, (2) the most frequent outcomes for those appeals, (3) the most prevalent challenges to digital forensics, and (4) which appeals are the most likely to result in a affirmed or reversed decision for the defendant. Overall, attendees of this presentation will be well informed as to how digital evidence has performed in the United States Courts of Appeal and how the United States Courts of Appeal generally view the science behind computer forensics.

**Impact on the Forensic Science Community:** Though the use of computer forensics in criminal investigations has expanded in recent years, there is limited empirical evidence about the prevalence of the use of digital evidence in the court system and its impact on prosecutorial outcomes. One case review from Losavio and Losavio examined cases that involved ineffective assistance of counsel with regard to computers as the basis for appeal from 2015–2016.<sup>1</sup> In addition, Cole, Gupta, Gurugubelli, and Rogers examined 100 appeal cases that involved digital forensics, and results revealed search and seizure was the main reason for appeal.<sup>2</sup> The current study aimed at further examining digital forensics in the United States Courts of Appeals. Specifically, this presentation will impact the forensic science community by an examination of cases from the United States Courts of Appeals in which legal issues were related to digital evidence. The purpose of this research was to determine the most common legal basis for appeals relating to the introduction or exclusion of digital evidence, the frequency with which cases involving an appeal regarding digital evidence affirmed or reversed for the defense, whether certain challenges to digital evidence are more prevalent than others, and whether there are trends or areas of the law as applied to computer forensics and digital evidence needing further attention by the criminal justice system.

This analysis was based on a review of cases in the United States Circuit Courts of Appeals from 2010 through 2015. Cases were identified via LexisNexis, using the following search terms: Computer, Computer Forensics, Chat Log, Electronic Evidence, Cell Phone, Sexting, iPhone, Child Pornography, Digital Evidence, Computer Investigation, GPS, and Encryption. The United States Circuit Courts of Appeals were an ideal venue for this study for two principal reasons. First, the 11 Circuit Courts of Appeal and the associated 94 United States District Courts cover the nation. There is at least one court in each state and the District of Columbia. Second, the 11 Circuit Courts of Appeal and the 94 District Courts adhere to the same rules of evidence—the Federal Rules of Evidence.

Probable Cause was the most frequently occurring legal issue encountered as a basis for appeal at 30.61%, followed by Sufficiency of Evidence at 28.57%; Defective Warrants at 12.24%; and Warrantless Seizures at 6.12%. Other legal issues less frequently encountered included Scope of the Warrant, Probative Value, Expectation of Privacy, Scientific Merit, Exclusionary Rule, Relevancy, Authenticity, and Hearsay.

Of the 147 cases included in this study, only 22 appeals were based on the science of computer forensics, including probative value, authenticity, hearsay, relevancy, and scientific merit. In each of those cases, previous rulings were affirmed. Out of the ten reversals for the defense, five were based on sufficiency of evidence. In the two cases where judgement was affirmed for the defense, one was based on a defective warrant, while the other was based on the scope of the warrant. Results will be compared to previous literature regarding digital evidence in the courts.

The majority of challenges seen in this study were based on Search and Seizure issues, rather than the science of computer forensics. This study only examined cases heard by the United States Courts of Appeals. Future research may involve an in-depth look at cases at the federal district court level to examine this issue.

One area of concern is particularity in regard to the scope of search warrants. Particularity governs how far the government can search, based on a factual predicate. Several important cases with regard to particularity have been decided by the courts recently (*Riley v. California* and *United States v. Ganas*).<sup>3,4</sup> This issue will likely continue to be central to appeals based on the Search and Seizure of digital evidence.

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#### Digital Evidence, United States Courts of Appeal, Appeals





## D1 Amusement Park Accidents Caused by Fatigue Failure

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**Learning Overview:** After attending this presentation, attendees will understand the mechanism underlying two amusement park accidents caused by fatigue failure of mechanical parts of the rides.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing failure mechanisms of amusement park rides.<sup>1</sup>

This presentation introduces two cases of amusement park accidents. One is a bungee drop accident caused by the fatigue failure of rolling bearings. The other is the “Crazy Clown” accident caused by fatigue failure of fastening bolts. In the bungee drop accident, a moving frame with 15 passengers stopped abruptly in the middle of the main structure at a height of 27m. While the rescue team was trying to rescue the passengers from the moving frame, it suddenly dropped, causing one rescue worker to fall to his death. A second accident happened nine years after the first accident. The moving frame with 12 passengers dropped abruptly as it was moving upward. It stopped at a height of 53cm due to the operation of the emergency brake system. Some passengers suffered spinal fractures. After an investigation, this study concluded that the moving frame was in an unstable equilibrium that would be broken with a small disturbance load while the rescue team was in operation. The unstable equilibrium state was maintained by the weight of the passengers and the frictional forces caused by the urethane wheels and poor lubrication of the pneumatic rod. The urethane wheels were severely worn out, and some were octagonal as a result. The rolling bearings of urethane wheels failed by flaking and corrosion that added frictional force. The moving frame was tested by varying the compressed air pressure as well, and all of the test cases were recorded by a video camera to analyze the time difference of the moving frame at each operation step. It was also concluded that the moving frame dropped because only one air compressor was operating while the other air compressor was not operating at the time of the second incident. It was recommended that a control system for the bungee drop should be installed to prevent the start-up of the moving frame, given the poor state of the compressed air system.

In the Crazy Clown accident, the fastening bolts of the rotating arm were broken abruptly when it turned around the central axis. The passenger was injured by the dropped cabin that was attached to the arm. After an investigation, the broken bolt was determined to have failed by fatigue as beach marks were commonly shown on the broken surface; however, the mechanical properties satisfied the requirements. From this, it was concluded that the fatigue was caused by external factors, such as improper fastening.

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### Amusement Park Accident, Fatigue Failure, Flaking

### D2 The Role of the Dynamometer in the Analysis of Manner of Death by Asphyxia: A Case Report and Review of the Literature

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**Learning Overview:** After attending this presentation, attendees will understand the role of the dynamometer in the study of cases of asphyxia.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the applicability of the dynamometer for studying the entity of the force and for the correct analysis of dynamics in cases of asphyxia.

Asphyxia is the respiratory failure caused by stopping the passage of air in the respiratory tract. It includes ligature strangulation that requires the use of a tool such as a cord applied around the neck. The strangulation includes the application of a force, a vector with a point of application, direction, and intensity. These characteristics can be measured by using the dynamometer, a device consisting of a spring and a graduated scale. In forensic literature, studies concerning the dynamometer are not reported. In this work, the dynamometer was used to reproduce an experimental model useful for studying the manner of death in cases of asphyxia.

In this case, a woman reported her husband for trying to strangle her by using an electric cable. The woman said she had placed her hands between the cable and her neck in an attempt to defend herself. From the statements, it emerged that during the struggle, both fell on the floor. The man confessed what had happened, but declared that he had never intended to kill her and that he had not exercised intense traction on her neck. The investigators collected the testimonies and carried out the analysis of the scene. The cable was seized and examined. The victim was analyzed with an examination of anthropometric measurements. The investigators reproduced an experimental model using a cable similar to the one seized. The cable was configured by reproducing a knot with a rope. The circumference was inserted around the neck of a dummy with characteristics similar to human skin. The free end of the cable was attached to a Crane Scale® digital dynamometer, capable of measuring the force in kilos (kg) on the display. The operators (both male and female) proceeded to: (1) tensile tests of the dynamometer at increasing intensity until maximum individual strength was reached; (2) evaluate the interaction between the cable and the surface of the dummy; (3) vary the application force both in intensity and in direction; (4) pull the cable, placing the knot on the lateral side of the neck (both anteriorly and posteriorly, then laterally to the right and left); and (5) simulate the tight constriction of the loop by interposing the hands of the dummy between the rope and the neck, according to the dynamics reported by the woman.

The cable had a diameter of 8mm and a rope with a knot showing blood stains. The inspection of the victim revealed some ecchymosis only on the left and back side of the neck. The victim had no other injuries except for bruising on her wrists.

The model showed that by pulling the cable, it was possible to restrict the circumference of the loop and compress the neck, easily reaching 5kg of force (minimum 2kg, max 25kg).<sup>1</sup> The cable caused a blunt tangential action on the tissue. The position of the knot on the lateral side of the neck caused a tight constriction mechanism of the loop on the posterior area of the neck, compatible with the victim's injuries. The experimental study showed that, even with the hands below the cable, it was possible to cause an intense traction. Finally, the position of the victim lying on the floor increased the negative effects of tight constriction. The manner was therefore compatible with a strangulation occurring by tightening the cable with the knot on the left neck area. In literature, four variables have been identified that have an interaction with the outcome of a strangulation: position, direction, force intensity, and surface of application.<sup>1-3</sup> In this case, the dynamometer allowed the estimation of the force applied on the neck and the study of the injuries caused on the tissue by analyzing potential measurable combinations in relation to the testimony and to the injuries analyzed. The data were used in court as scientific support to demonstrate the homicidal intent of the perpetrator and the reliability of the victim's statements.

The dynamometer offers the advantage of: creating reproducible models at low cost; studying the effects of forces pre-established by the operator; reproducing different dynamics; and verifying the witnesses.

It would not be applicable in suffocation and strangulation by the hands in which there is not a tool on which to apply a traction and, therefore, to measure the force.

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#### Forensic Sciences, Asphyxia, Dynamometer



### D3 Railway Fatalities: The Importance of the Autopsy for the Reconstruction of Railway Accidents

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**Learning Overview:** After attending this presentation, attendees will better understand the possibility of reconstructing the dynamics of a railway accident through a multidisciplinary approach.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining the multidisciplinary approach of reconstructing a railway accident, in particular by observing and studying, during the external examination of the cadaver, the specific physical imprint abrasions, by inspecting the railway vehicle and the areas surrounding the railway track and, in some cases, through autopsy, which can reveal multiple lesions and injuries not found only by external examination of the cadaver. This presentation, showing two unusual railway accidents, will also impact the forensic science community by enhancing knowledge of the reconstruction of a train accident (rail accident or train crash).

The train accidents involved heavy vehicles with metal wheels and prescribed tracks on rails (trains, subway, or underground, tram, etc.), which cause serious and complicated injuries achieved during the phases of impact from knocking down and sometimes running over a person, almost always having fatal detrimental effects.

**Method:** Two railway accident cases are presented here. The first deals with a 30-year-old man, found in a prone position, along the ballast of the inter-track section of the Bari-Lecce railway route. During the cadaverous external examination of the victim, a circular ecchymotic-excoriate with a diameter of 13.5cm was observed in the left posterior dorsal region that corresponded in morphology and dimensions to the right buffer of the railway locomotive, previously examined during the inspection. The injury was characterized by a large laceration caused by the skin being stretched as a result of the full-scale impact. That was sufficient to establish that the victim, at the moment of the adverse event, had his back toward the locomotive.

The second case deals with a 39-year-old man, found in a supine position along the ballast adjacent to the railway track of the Bari-Lecce route who, while fleeing from a robbery, jumped over a protective wall of the Ferrovie dello Stato Italiano (Railroad of the Italian State), and fatally collided with a railway convoy, causing injuries that at the first external examination appeared to be caused solely by falling from the top of the wall. Later, however, the autopsy showed very serious injuries to the head, characterized by a wide fracture and complete detachment of the brain from the brain stem, the ribs, including several rib fractures, and lesions of the left lung, the abdominal, with right hepatic and renal laceration, and the limbs, including a fracture of the head of the left humerus and multi-fragmentary fracture of the left femur. This injury could be caused by a violent impact against protruding parts of the convoy and by the body being thrown toward fixed surrounding structures as can be seen from the frontal part of the cranium where the presence of an ecchymotic and excoriate rectangular lesion of 10 x 16cm with a series of imprint abrasions close together inside it was identified, with thin even dots like a “barcode,” all attributable to the morphology and size of a bump against the stem of an old track located on the ballast where the body of the victim was found.

For a reconstruction of the dynamics, a careful external examination of the cadaver and an inspection of the railways were insufficient in the first case; in the second case, it was necessary to consider the autopsy assessment.

This study revealed the significant impact of the multiple injuries related to the rail accidents. The goal of this presentation is to show that linking together the inspection of the railway vehicle, the surrounding areas, the external examination of the cadaver, the observation of typical imprint abrasions to the body, and the autopsy is extremely important for reconstructing the dynamics of the train accident.

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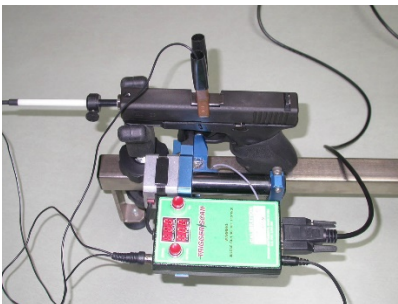
#### Rail Accidents, Physical Imprint Abrasions, Train Collision

### D4 A Comparison of Scientifically Valid and Traditional Firearm Trigger Mechanism Evaluation Techniques

*John Nixon, MBA\*, ARC, Bippus, IN 46713*

**Learning Overview:** After attending this presentation, attendees will have gained an appreciation of the basic science and technology behind firearms trigger mechanism evaluation, current trigger mechanism evaluation techniques, the influence that test techniques and their results have on final conclusions drawn, and the ultimate impact of those conclusions within the criminal and civil justice systems. Attendees will learn that widely applied crime laboratory trigger examination techniques generate inaccurate and incomplete data, and that they frequently result in misleading conclusions being drawn with regard to firearm safety. Attendees will be introduced to a scientifically valid trigger mechanism evaluation technique that has been adopted by many crime laboratories and firearms manufacturers worldwide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of a scientifically valid trigger testing technique and associated data analyses. The implications for civil and criminal litigation, where accidental or negligent discharge of a firearm is an issue, will be discussed.



The effort required to discharge a firearm is a function of both the force applied to and the distance traveled by the trigger. Engineers have long been able to design trigger mechanisms and calculate the travel, peak force, and total effort required to discharge a firearm. Engineers have had the capability to scientifically test trigger mechanisms to determine the force-travel profile, and calculate the effort required to actuate the mechanism. However, until relatively recently, the techniques involved were labor intensive and required a laboratory. Analysis and interpretation of the test data required appropriate engineering and mathematical education and training.

In the absence of an engineer and a laboratory, firearms users and armorers wanted a simple way to evaluate “trigger pull” and, historically, the arsenal weight and spring gauge techniques have been used to determine the peak force required to actuate a trigger. These peak force techniques generate minimal and inconsistent data that are misleading when used to quantify the relative ease of firearm discharge.

A purpose-built electro-mechanical trigger testing device linked to a computer, and utilizing analytical software, makes relatively easy work of scientifically valid trigger mechanism testing and evaluation. The technique, equipment, and data analyses are described and explained with the aid of practical examples and case studies. Specifically, the firearm is secured in a test rig, and an electrically driven mechanical arm moves the trigger rearward at constant velocity. The force range is from 0 to 20 pounds, with a resolution of 0.007 pounds, and the travel range is from 0 to 1.4 inches. Every 0.005 inches, the electro-mechanical test apparatus software records the force exerted on the trigger face, and these data are downloaded to a computer where the software plots a chart and utilizes integral calculus to determine the total effort required to actuate the trigger mechanism. The software displays key trigger characteristics, such as energy to actuate and trigger travel to actuation. A suitably qualified person can use the chart to visually identify potential defects, modifications, and/or design deficiencies in the trigger mechanism, and the captured data may be exported and analyzed in greater detail. The equipment can be further modified with the addition of an electrical circuit that enables the determination of lock time (illustrated). Another advantage of this system is that, for practical purposes, the effort required to actuate the trigger mechanism is independent of the position of the test arm on the trigger face.

It is concluded that widely employed “traditional” peak force methods of firearms trigger evaluation should be phased out and replaced by electro-mechanical trigger evaluation, with the measure of ease of discharge expressed in terms of the effort required to actuate the trigger mechanism, thereby providing a scientifically valid method of comparison between firearms.

#### Trigger Pull, Trigger Actuation Energy, Accidental Discharge



## D5 Defect Investigation of a Gun Rack

*Darren Franck, MSME\*, Advanced Engineering Associates, Inc, Charleston, WV 25304; Harold Franck, MSEE, Advanced Engineering Associates, Inc, Charleston, WV 25304*

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**Learning Overview:** The purpose of this presentation is to highlight investigative methods to address untested hypotheses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting investigative methods to assess the validity of a defect claim. These methods will be compared to untested hypotheses promulgated by opposing experts, which highlights the need for ethical standards.

This case study presents a defect investigation involving a manufacturer of a gun rack used in a police cruiser. Civil actions were brought against numerous parties, including the installer of the gun rack, manufacturers of components used in the gun rack design, and the owner of the cruiser. The defect allegations were supported by multiple experts, who opined that the design and installation of the gun rack was defective.

The incident occurred during a training course for members of a local police department. The plaintiff was seated in the right front passenger seat of a police cruiser during braking tests. It was alleged that the gun became dislodged from the rack as the vehicle decelerated, which caused the butt end of the gun to strike the plaintiff's head. Severe and lasting injuries were claimed, which formed the bases of the lawsuit.

The plaintiff's experts presented various hypotheses to explain the alleged failure of the gun rack. The rack includes a pocket to secure the butt end of the gun, a trigger guard to prevent accidental discharge of the weapon, and a locking mechanism at the barrel end. The installation did not include the trigger guard at the request of the vehicle owner. One expert hypothesized that the absence of the trigger guard caused or contributed to the dislodgement of the gun. Another expert claimed that the unsecured mount of the timer module produced a current spike that unlocked the mechanism and allowed the gun to detach from the mount.

None of these experts performed pertinent testing of the gun rack assembly. The failure to test their hypotheses represents a disregard for the scientific method and a violation of ethical standards. Experts were hired by one of the defendants to assess the validity of the defect claims. Numerous tests were conducted by the experts involving exemplar gun racks subject to inertial loads, vehicle decelerations, vibrations, and impact loads. Measurements were gathered to document loading on the locking mechanism, decelerations on the timer module, as well as data culled from current and voltage probes.

None of the tests resulted in failure of the locking mechanism. Vehicle decelerations and impact loads did not cause a current spike sufficient to open the lock. Moreover, manual depression of the push button on the timer module during hard braking did not yield detachment of the gun from the locking mechanism. Static testing confirmed that a significant inertial load would be required to open the lock spring even after depressing the push button. These loads were not achieved by the deceleration forces and weight of the gun. Moreover, the allegations are not consistent with the movement of the gun resulting from collapse of the butt stock. Similarly, the installation of the trigger guard would not have affected the detachment of the gun from the rack. However, the alleged movement of the gun to the front dashboard would have been highly unlikely in the absence of the trigger guard.

The experts' work allowed their clients to be released from this lawsuit. However, the suit involving the remaining defendants continued, resulting in unnecessary legal and expert fees. This presentation highlights the need for ethical standards in forensic sciences.

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### Locking Mechanism, Non-Destructive Testing, Ethics



## D6 The Effect of Fabric Tension on Knife and Tool Damage

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**Learning Overview:** After attending this presentation, attendees will understand how fabric tension can affect the damage caused by the penetration of knives during stabbing and slashing events.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing new data that can be used to better understand the effect of fabric tension on the damage patterns left during a knife attack.

Knife crime continues to be a major challenge in the United Kingdom where access to guns is heavily reduced compared to the United States. In 2019, by May 14 there had already been 100 fatal stabbings in the United Kingdom, not counting the events that caused life-changing injuries.<sup>1</sup> However, this issue is not solely a problem affecting the United Kingdom. The United States has a higher homicide rate “due to knives or cutting instruments” per every million of population; in 2016, this was at 4.96 for every million of population compared to the United Kingdom’s 3.26 per million.<sup>2</sup>

Damage to clothing regularly occurs during a sharp-force fatality. Karlsson reported that 79% of 145 victims had damage to clothing after being subjected to a sharp-force event.<sup>3</sup> Examination of the resultant size and appearance of the severance in the fabric post-impact can provide information about the characteristics of the weapon used, the dynamics of the assault, and the post-impact conditions to which the fabric was exposed.<sup>4</sup> The tension applied to a fabric is affected by clothing fit (i.e., ranges from ill-fitting clothing that is oversized or to purposely undersized such as athletic sportswear). Tension applied to a fabric reportedly influences the severance profile; however, there is very little experimental data available to provide confidence in the analysis of what the severity of the influence might be. Cowper et al. provides one of the few studies in this area.<sup>5</sup>

The goal of this research was to investigate whether fabric tension affects the resulting severance due to stab and slash events. A range of commonly worn fabrics were pre-tensioned over silicone skin-simulant resin. The pre-tensioning ranged from loose fit to tight. Several sharp implements, including kitchen knives and sharpened screwdrivers, were used to perform stabbing events that were recorded using high-speed imaging. The forces generated during the stab events were recorded using an instrumented knife and a dynamometer. The fabric was examined post-test to determine the effect of tension on the severance. The results are presented in a way that provides insights that can be used to inform future analysis of fabric damage.

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### Knife Crime, Fabric, Tension



## D7 The Effectiveness of Overt and Covert Protection Against Attacks by Sharp Implements

*Sarah V. Hainsworth, PhD\**, Aston University, Birmingham B4 7RT, UNITED KINGDOM; *Patrick H. Geoghegan, PhD*, Aston University, Birmingham B4 7ET, UNITED KINGDOM

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**Learning Overview:** After attending this presentation, attendees will understand how weapon profile affects the penetration ability of knives and spikes in stabbing and slashing attacks against body armor and slash hoodies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how overt and covert protection performs against attacks from knives, shivs or spikes, and other sharp implements.

Kevlar® is commonly used in body armor to protect against stab, slash, and spike attacks. Unlike ballistic attacks, attacks with edged and spiked weapons usually occur with the assailant and intended victim in close proximity to each other (unless the weapon is thrown). Stab/slash vests and clothing are therefore commonly worn by police officers, prison officers, security guards, and doormen. Kevlar® armors are typically lightweight in comparison to other body armors so the vests can be worn for long periods without undue fatigue or drop-off in performance of the wearer. Kevlar® vests can have both stab and spike ratings and protect against weapons such as knives, broken bottles, and syringes, for example. In prisons for example, protection is required from homemade weapons or “shivs.”

Protection for individuals can be in the form of stab-resistant protection worn over clothing (overt protection) or panels worn under normal clothing (covert protection). A range of different types of clothing with Kevlar® in the weave of the clothing is also available from hoodies to sweaters and T-shirts. There are a number of standards available for body armor. Commercially available clothing usually complies to the National Institute of Justice (NIJ) Level 1 stab and spike resistance (CAST/HOSDB KR1 and SP1). However, composite manufacturers and other protective materials are continuously developing new materials that offer new protection mechanisms.

The goal of this research is to show how weapon geometry interacts with stab-proof materials using high-speed video testing of attacks by sharp implements and subsequent analysis of fabric damage using microscopy. The outcomes will be used in collaboration with manufacturers of anti-stab clothing to show how performance of these materials and clothing can be improved. The stab-resistant clothing and protection will be contrasted with tests against ordinary fabrics. The tests show for a range of weapon types how a stab vest worn over clothing, a covert anti-stab panel, a slash hoodie, and a long-sleeve T-shirt perform in response to penetration by a weapon. The forces generated during the stab attacks will be recorded using an instrumented knife and a dynamometer. By understanding the exact mechanisms of damage to the materials and how the implements penetrate through the material, this study will be able to inform the development of stab-proof vests and clothing and allow improved personal protective equipment to be developed.

The results show that knives generally penetrate the fiber network by cutting through the individual fibers but that shivs and other pointed instruments penetrate through the fiber network and, therefore, differing strategies are required for providing protection for different weapons.

The results are discussed in terms of the way in which these insights can inform the future development of body armor.

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### Stabbing, Body Armor, Knives



### D8 The Impact of Ventilation on Fire Patterns in Full-Scale Structures: Experiments, Analysis, and Education

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**Learning Overview:** The goals of this presentation are to: (1) develop a better understanding of the relationship to the availability of oxygen in a structure fire and how that impacts the fire damage; and (2) learn how to access the data and video results from the study via a freely available, interactive web portal.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the report, the time histories of the data, and the videos from this study that provide foundational documentation for the understanding of ventilation-controlled fires and the resulting fire patterns. Lack of knowledge of post-flashover and ventilation-controlled fire damage by fire investigators has resulted in unwarranted prosecutions and incarcerations for arson. This study supports the understanding of separate and distinct fire patterns that are generated by ventilation-controlled burning conditions in a structure. In past criminal cases, fire investigators have misunderstood ventilation-generated patterns and incorrectly identified them as evidence of arson.

Residential fires tend to be ventilation limited. Understanding ventilation-limited fires can aid fire investigators in analyzing fire patterns and locating the area of origin. When and where the fire receives oxygen impacts the fire and subsequent fire patterns. With the support of the National Institute of Justice, a series of full-scale fire experiments were conducted to: (1) examine how differences in ventilation to full-scale structure fires result in changes to the fire damage and fire patterns within the structure; (2) measure the fire environment within the structures and compare the data with the fire damage in the structures; and (3) document the repeatability or lack thereof of the fire conditions and fire patterns within a structure based on the available ventilation. The experiments were planned with the assistance of a technical panel that included members of the American Academy of Forensic Sciences, Bureau of Alcohol Tobacco and Firearms, International Association of Arson Investigators, National Association of Fire Investigators, National Association of State Fire Marshals, National Institute of Standards and Technology (NIST), NIST Organization of Scientific Area Committees for Forensic Science, and the National Fire Protection Association.

The test structures included a 1,200 sq. ft. one-story ranch structure and a 3,200 sq. ft. two-story colonial structure. The colonial had an open floor plan with a two-story family room and open foyer. The test scenarios ranged from fires in the structures with no exterior ventilation to room fires with flow paths that connected the fires with remote intake and exhaust vents throughout the structures. Elevated fires originating in the kitchens were also examined.

The structures were photographed before and after each fire experiment. Instrumentation was installed to measure gas temperature, gas pressure, and gas movement within the structures. In addition, oxygen sensors were installed to determine when a sufficient level of oxygen was available for flaming combustion in a given area. Standard video and firefighting thermal imaging cameras were also installed inside the structures to capture information about the fire dynamics of the experiments. Video cameras were also positioned outside the structures to monitor the flow of smoke, flames, and air at the exterior vents.

The results from a series of full-scale house experiments examining the impact that changes in ventilation had on the fire patterns are available for reference and further study on the free UL Firefighter Safety Research Institute (FSRI) web portal, [fireinvestigation.ulfirefightersafety.org](http://fireinvestigation.ulfirefightersafety.org).

This presentation will highlight key findings of the study using photos, data, videos, and fire flow graphics. The use of the fire investigation portal to access the report, data, videos, and free on-line training will also be demonstrated.

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#### Fire Pattern, Ventilation-Limited Fire, Flashover





### D9 The Explosive Destruction of a Horse Trailer With Integrated Living Quarters

*David R. Bosch, PhD\*, Forensic Engineering Inc, Phoenix, AZ 85008; Mark C. Pozzi, MS\*, Sandia Safety Sciences, Edgewood, NM 87015*

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**Learning Overview:** The goal of this presentation is to share with the forensic science community the detailed methodology used to determine the root cause of an explosion and flash fire within a recreational vehicle that severely burned two victims.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a detailed discussion regarding the unusual failure mode of a sewer system commonly used in recreational vehicles. An example of the risks and costs associated with expectation bias will also be discussed.

The incident to be presented and discussed involved a horse trailer with integrated living quarters that was involved in a gas explosion, which resulted in two severely burned occupants. The trailer with living quarters was being used to attend an equine event by two female enthusiasts. At the time of the explosion, the two occupants were within the living quarters of the horse trailer when a fugitive gas ignited, causing the severe explosion and flash fire. The two occupants were able to self-extricate themselves from the burning trailer. Upon exiting the trailer, one of the occupants was on fire. Witnesses quickly placed her on the ground and extinguished the flames. Both of the occupants suffered serious burn injuries.

The trailer and living quarters were severely damaged by the explosion and flash fire, resulting in significant investigative challenges. A team of experts with specialized skills in fire/explosion origin and cause, gas migration and gas explosion energy, mechanical and materials engineering, Recreational Vehicle (RV) codes and standards, and gas detection technology were quickly retained. The initial investigation revealed that the trailer was equipped with Liquefied Petroleum Gas (LPG) -fired appliances, which included a gas refrigerator, gas furnace, gas stove top, and a gas water heater. The entire LPG system, including all piping, fittings, controls, and appliances, were inspected and tested for leaks multiple times. After failing to find the source of the fugitive gas in the LPG components, the investigative team chose to consider the possibility that sewer gas from the onboard sewer system could have been the source of gas that exploded. After obtaining additional discovery and completing additional investigation, interviews (including the finding that there had never been a case involving a sewer gas explosion in a RV), and testing, the investigation ultimately revealed that the explosion and resulting conflagration were the result of errant sewer gas (i.e., methane). This finding was consistent with the discovery documents, which revealed that the black water holding tank had been replaced after having been torn from the trailer during an accident. The cause of the sewer gas leak was determined to be the failure to properly install the black water holding system, which allowed sewer gas to migrate into the living quarters of the trailer where the sewer gas mixed with air, ignited, and exploded. If the black water holding system had been properly installed, there would not have been an explosion and flash fire and the two female occupants would not have been seriously burned.

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#### Gas Fire/Explosion, Recreational Vehicle Fire/Explosion, Sewer Gas

### D10 An Investigation Into the Cause of a Fire Engulfing Two Collided Buses

*Qiang Chen, PhD\**, Chinese Automotive Technology Research Centre, Beijing 100070, CHINA; *Xiao Hu\**, Beijing 100070, CHINA

**Learning Overview:** After attending this presentation, attendees will understand how a fire started and its origin after two buses collided accidentally. Attendees also will get a better view of a magnetic-steel remanence detecting method and metallographic method as applied to the research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility of a magnetic-steel remanence detecting method and metallographic method as a combined technique when carrying out vehicle-fire analysis.

**Background:** This case involves two urban buses both packed with more than ten passengers. When the two buses were traveling along a side road in the opposite direction, they had a head-on collision when passing each other. Only a few of the passengers were evacuated promptly and a dozen people got stuck between distorted seats. Within minutes, both buses burst into flames. Eventually, the buses were burned down to the bare metal frames, causing heavy casualties. Considering its social impact, the local government administrative departments required a forensic investigation into what had started the fire.



**Methods:** First, the overall appearance of the two buses' remains were checked to determine the approximate areas of the worst burning. In this operation, forensic engineers should pay particular attention to the colors of residual metal parts, which would tell the developmental trail of the fire. Second, electrical wires, fuel pipelines, and other combustibles around the possible epicenter of the fire were analyzed. As the previous analysis shows that the earliest flame started near one of the drivers' dashboards other than its mid-tank or rear-engine, forensic engineers should focus on examination of cables. Third, residual cables were tested with total coverage by magnetic remanence detector, and circuit melted beads were looked for. Fourth, samples of circuit melted beads were examined through metallographic analysis apparatus, then these microstructure images were compared to find the primary short-circuited melted mark.



**Results:** A magnetic remanence detector revealed that the highest test value is 6.3mT, compared with the rest of the area of no more than 0.3mT. Two circuit melted samples were collected from the residual cables nearby. In the lab, two samples were carefully observed under metallographic microscope respectively. The microstructure images indicated that one sample has distinct characteristics of primary short-circuited melted mark. Thus it is concluded that the deadly fire was caused by a primary short circuit that would ignite a fire around combustible materials.



**Conclusions:** Electrical circuits in all vehicles have become more and more complex. In cases of head-on collision, a primary short circuit actually has caused a number of fire accidents without obvious traces. The results of this study confirm that metallographic analysis combined with magnetic remanence detecting could help forensic engineers investigate and locate the fire point efficiently and accurately.

#### Fire Investigation, Metallographic Analysis, Magnetic Remanence Detecting



## D11 Understanding and Controlling the Potential Risk of Jobsite Construction Hazards

*Daniel M. Honig, PE\*, Structures Consulting Engineers, Swarthmore, PA 19081*

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**Learning Overview:** After attending this presentation, attendees will understand the significant safety factors required to avoid and/or minimize jobsite construction hazards. The work hazards presented by preexisting subsurface site utilities in direct proximity to the installation of new equipment and related foundation construction will be identified and evaluated. The accurate field measurement and documentation tolerance requirements for the installation of new foundation work relative to existing subsurface conditions requires ongoing careful and redundant engineering and field supervision tolerances and back checks. Forensic engineering requires recognition of these hazards and taking appropriate precautionary measures since this is incumbent to prevention, work safety, and identifying related issues should an incident occur.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of the inherent jobsite hazards and risks when altering and/or upgrading existing electrical substations with new adjacent foundations. Given the limited timeframe needed to complete this type of industrial construction project, ongoing field engineering oversight, documentation, and quality control are critical to the successful and safe completion of this work.

In the summer of 2012 in an eastern Pennsylvania metropolitan area, a pharmaceutical company undertook a fast-track construction program for the replacement of existing switchgear equipment, including alterations to an adjacent electrical substation at one of their manufacturing facilities. For this work, they entered into a design/build fast-track agreement with an energy management company. Due to a large number of existing underground electrical ductbanks and related utilities within the foundation footprint of this project site, the designer/builder elected to proceed with the installation of a micropile foundation system. A general contractor was subsequently engaged in order to complete this overall substation and utility upgrade. The special and complex foundation system for support of this new building project and the civil/structural scope of work needed for completion of this project required the engagement and coordination of multiple subcontractors/subconsultants. This included the engagement of a consulting engineering firm for the field quality control of the new structural foundation system and building superstructure elements. During the exploratory excavation and installation phase of micropile drilling, the drill rig contacted and penetrated a ductbank containing an energized, high-voltage feeder cable. This incident caused a jobsite explosion resulting in the electric shock and serious injury of the drill operator.

This case study provides an engineering analysis of causal factors in this professional negligence case on behalf of the plaintiff (the drill rig operator). The significance of the resultant failure of this case illustrates how crucial diligent engineering analysis is for jobsite construction safety. Specifically, within the forensic community, recognition of these hazards is essential for identifying causal engineering factors for the prevention of construction failures.

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### Micropile, Drilling, Construction Safety

### D12 Improving the Accuracy of the Quantitative Method for Evaluating Fracture Load by Blunt Force: A Proposal for the Fracture Load Analysis Method Using a Victim's Bone Shape, Bone Mineral Density Distribution, and Soft Tissue Thickness

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**Learning Overview:** After attending this presentation, attendees will understand the advanced fracture risk evaluation method.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an advanced fracture risk evaluation method.

Restrictions on guns are strict in Japan, so many weapons used in murder cases are blunt weapons or knives. In the cases of murder and domestic violence using a blunt weapon, such as a hammer or hand, it is necessary to quantitatively evaluate the external load received by the human body and to objectively show the killing ability of the blunt weapon in court.<sup>1</sup> However, although there are established evaluation methods, such as head injury criterion for brain damage, there is no quantitative method for evaluating the fracture risk using a blunt weapon. Therefore, this study accurately calculated the load on the bone by striking the load cell covered with dummy skin with the blunt weapon, and the method for evaluating the fracture risk was established by comparing with the literature the value for the fracture load in every body part.<sup>2</sup> In addition, bone strength evaluation technology that accurately reflects bone characteristics, such as individual bone shape and Bone Mineral Density (BMD) distribution using computer simulation, has been developed and used in the medical field. It is considered that this technology could be used as an effective tool in fracture risk evaluation, and a quantitative method for evaluating fracture risk by static load, such as nursing care, has been developed. However, in the above two cases, it is not possible to quantitatively evaluate the fracture load by blunt force, such as the impact of the blunt weapon, considering the individual bone characteristics and the soft tissue thickness.

In this presentation, a quantitative method for evaluating fracture load by blunt force was designed and the method is reported. The outline of the method is shown below. The blunt force was applied to the load cell covered with dummy skin, and was converted to energy by measuring the propagation load with the load cell: the energy input to the dummy skin was calculated.<sup>2</sup> Then, the relationship between the input energy and the load that the bone received in each soft tissue thickness was clarified in advance: this is the logistic line of the load that bone received. The load that bone received was calculated by applying it to the input energy calculated for this logistic line. In the past, fractures were evaluated by comparing this load and the literature values for the fracture load, and the effects of bone characteristics in individuals have not been taken into consideration. On the other hand, fractures can be evaluated using the human bone model that reproduces the victim's bone characteristics in bone strength evaluation based on Finite Element Analysis (FEA). However, the buffer property of soft tissue cannot be reproduced during analysis. Therefore, this study combined these experimental and analytical methods. As a result, it was possible to analyze the fracture load by accurately calculating the load received by the bone using the above method and applying this load to the load condition in FEA for human bone model. The proposed method enabled fracture analysis using the victim's bone characteristics and soft tissue thickness.

Super soft urethane resin (EXSEAL) was used for dummy skin, and CLM-10KNS (TML) was used for load cell. As soft tissue, porcine soft tissue, which is a human substitute, was used. MECHANICAL FINDER (RCCM) was used for bone strength evaluation software using the finite element method. With this software, a bone model that accurately reflects BMD can be created by the quantitative computed tomography method.

A prototype has been completed to quantitatively evaluate fracture load by blunt force using the flow and equipment described above. Also, the fracture load  $F$  [N] is given by the following formula when a load is applied to the infant skull in the area  $A=100$  to  $1,000$  [mm<sup>2</sup>] in this study.<sup>3</sup>  $F=7.7615 A + 1786$ . Thus, it was clarified that the load loading area greatly affects the fracture risk. Therefore, aiming at the application of the load area to the load condition in FEA, the load area that the bone receives is measured experimentally. In addition to experimental verification using this protocol, it is applied to the actual appraisal.

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#### Fracture Risk Evaluation, Individual Bone Characteristics, Soft Tissue



### D13 Physical Properties of Additive Manufacturing to Combat the Illicit Use of 3D Printing Technology

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**Learning Overview:** After attending this presentation, attendees will understand what physical factors of a 3D-printed object are significant for their forensic discrimination and classification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying systematic differences in the overall dimensions of a 3D-manufactured part due to the brand/type of printing machine, polymer filament material, software used to generate the printed file, and orientation of the printed part.

3D printing is becoming easily affordable and readily available. Available since the 1980s, 3D printers are used to create a range of objects, such as airplane and automotive parts, footwear, and medical prosthetics. The easiest way to begin the process of 3D printing is by using an existing computer model, which are available through open-source file-sharing sites. Criminals who take advantage of this technology can manufacture counterfeit parts, Automated Teller Machine (ATM) skimmers, firearms or their parts, and improvised weapons by the modification or replication of open source files. Additionally, there is little regulation for the manufacturing and ownership of 3D guns, which creates challenges in identifying parts and materials. The ability to associate a 3D-printed part to the printer that made it or the polymer from which it was made has the potential to be valuable in a forensic investigation. However, forensic research in additive manufacturing is lacking and, therefore, there are insufficiently validated test procedures.

The long-term goal for this research is to be able to associate a printed part with its source (e.g., the printer or the polymer). The potential for this type of association is based on the reproducible differences between different printers combined with consistency in the manufacturing of parts from a single printer. This research specifically evaluated the effect that different manufacturing parameters have on the physical dimensions of the resulting 3D-printed object. The manufacturing parameters that were tested included using different printers (same and different brands), polymer filament material, software used to generate the printed file, and orientation of the printed part. The hypothesis is that there are systematic differences at the macro scale that would enable differentiation of printed parts manufactured using different printers and parameters.

The MakerBot Replicator 5<sup>th</sup> Generation, MakerBot Replicator Plus, Stratasys Dimension Soluble Support Technology (SST) 1200es, and Sincor 3Dwox were used to create rectangles, cylinders, and wedges using Acrylonitrile Butadiene Styrene (ABS), Polylactic Acid (PLA), and Tough PLA Material. Programs such as SolidWorks, and Fusion 360, AutoCAD were also tested on the 5<sup>th</sup> Gen, Plus, and Dimension. Measurements of mass and dimensions (x, y, z, inner and outer diameter) were taken of each generated part. The Analysis Of Variance (ANOVA) of the measured physical measurements indicated there are statistically significant differences (p-value less than 0.05) between 3D-printed parts that are manufactured with different printers, software, orientation, and material. As the database increases in size, there is a possibility of determining the discriminating potential of physical measurements for the identification and categorization of 3D printing characteristics, which ultimately has the potential to help trace a printed part to its source.

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**Additive Manufacturing, 3D Printing, Open Source Files**



## D14 Hot-Air Ballooning—Rare as Disastrous Incidents: An Italian Case Report

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**Learning Overview:** After attending this presentation, attendees will understand the characteristic injury patterns of hot-air balloon crashes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that identification of patterned lesions by the forensic pathologist can help the understanding of the dynamics of events.

Hot-air ballooning incidents are relatively rare; however, when they do occur, they are likely to result in a fatality or serious injury.<sup>1</sup> Human error is commonly attributed as the cause of hot-air ballooning incidents.

Hot-air balloon baskets are not enclosed and serious injuries may result from blunt trauma as a result of a fall or burns/electrocution resulting from collision with power lines of fuel leaks, so the nature itself of hot-air ballooning means that there is high potential for serious injury during ballooning incidents.<sup>2,3</sup>

In October 2015, during the Matera Balloon Festival, a hot-air ballooning pilot (72-year-old man) and two high school students (18 and 20 years old) crashed with a powerline while flying over a hill. A small onboard fire started after the impact and the vehicle lost altitude. The young men jumped from the basket. Their bodies were found 500 meters apart. The pilot remained on the basket and survived with some burns.

Computed Tomography (CT) scan examinations, autopsies, and histological analyses were performed on the two decedents. In the older student, the CT scan exam showed multiple fracturing (skull, cervical, chest, sacrum, hip, heels). The external examination revealed burns on the vest (anterior surface). Hair, eyebrows, beard, and mustache were burnt and there were multiple skin abrasions and lacerations on the entire body. Some skin blisters were found on the upper limbs. The autopsy revealed the presence of fracturing complexes of skull and chest with hemorrhagic infarction of soft tissue. There was hemothorax and the lungs were lacerated. In the abdomen, there were about 300cc of blood. Liver, spleen, and kidneys were lacerated and the hip was fractured. The histological analysis confirmed the burn lesions on the upper limbs' skin and tissue damages in the other organs.

The younger student's CT exam showed fractures of the skull, facial bones, chest, cervical-dorsal-lumbar-sacral rachis, and hip. The vest and the T-shirt of the student were burnt on the right lateral surface. The hair was burnt and the face had a large contusion area with some skin lacerations. The entire body had multiple abrasions and lacerations. The cadaveric section demonstrated complex fracturing of the skull and chest with lacerations of the lungs, heart, liver, and spleen. The histological studies confirmed the damage of the organs and the burn damage on the skin of the right upper limb and hip.

The cause of death of both young men was a traumatic-hemorrhagic shock by precipitation.

In conclusion, this case provides many topics for thought. First, the hot-air balloon crashed with a powerline and this event agrees with many international studies that show how the causes of hot-air balloon incidents are commonly associated with the human error of the pilot. Second, multiple patients with severe injuries can result from a single balloon crash. In this case, all three passengers suffered the consequences of the incident, although in different measures. Third, the severity of injuries may vary widely, from contusions to burns and death. In the crash at the Matera Balloon Festival, the only survivor exhibited only some burns while the deceased passengers were found to have burns, multiple fracturing, several organ lacerations, and both died from traumatic hemorrhagic shock.

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### Hot-Air Ballooning, Incident, Trauma



## D15 Complexity vs. Uncertainty in Models

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**Learning Overview:** After attending this presentation, attendees will be able to discuss the trade-offs between complex models requiring many parameters to be estimated versus the uncertainty associated with the parameters required in complex models.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by making practitioners aware of the pitfalls of overly complex mathematical models.

With the advent of high-performance computing, there has been a tendency to build models that are more and more complex. This is exacerbated by the ability to generate very large data sets using remote sensing, high-speed telemetry-using cell phone networks and similar data networks, and the prevalence of Geographic Information Systems (GIS) to process these data.

In environmental settings such as groundwater modeling where the parameters in these models are uncertain, complex models can result in very large predictive uncertainty. Parameter estimation programs such as BeoPEST can be used to estimate a large number of parameters quite effectively, thus reducing the predictive uncertainty. However, depending on the observation data set, there may be a substantial parameter null space that cannot be estimated.

This presentation provides a review of groundwater models used in litigation over the past 30 years. Many of these models have been used in Interstate Compact litigation and have been the topic of disputes before the Supreme Court of the United States. This presentation explores the complexity of the models and the methods applied to reduce the uncertainty of the model. In particular, this presentation reviews the increase in complexity over time and attempts to answer the question of whether the increased complexity leads to increased or decreased uncertainty in the predictions of the models. In a litigation environment, there is the additional element that the trier of fact may not be well versed in modeling, calibration, and uncertainty, and yet the outcome of litigation where there are competing models may depend on a decision selecting one model over another.

Occam's Razor in a modeling context may require that instead of using very complex models, we ask more fundamental questions. For example, can the question be resolved by targeted measurements, or by a water budget analysis, or using a simpler analytical solution, or perhaps using a change model, as opposed to building a complex model. To what extent does a simpler approach result in predictions that are as reliable as a more complex model?

This presentation demonstrates that complexity does not necessarily translate to greater certainty and, particularly in a litigation context, how complexity often calls into question the results of a more complex model.

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### Complexity, Uncertainty, Models



### D16 Bulk and Micro-Scale Trace Element Analysis of Glass Standard Reference Materials (SRMs) Using Modern Nuclear Analytical Methods and Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS)

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**Learning Overview:** After attending this presentation, attendees will understand the importance of fit-for-purpose National Institute of Standards and Technology (NIST) SRMs for calibration of instrumentation for the forensic analysis of glass samples. The goal of this project is to provide the forensic science community with additional data about the suitability of currently available NIST SRMs for micro-scale analyses. This effort will also generate new concentration values for trace elements in existing SRMs. Additionally, the developed techniques for bulk and micro-scale analysis will be used to certify new trace-element glass reference materials specifically designed for use in forensic practice, such as the Organization of Scientific Area Committees (OSAC)-approved American Society for Testing and Materials (ASTM) E2927-16e1 standard test method for analysis and comparison of glass evidence.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community's understanding by providing additional information on currently available NIST SRMs, such as highlighting elements affected by potential heterogeneity biases and providing additional trace element abundance data.

Analysis of glass using micro-sampling techniques, such as micro X-Ray Fluorescence ( $\mu$ -XRF) and LA-ICP/MS, is a key capability for many forensic laboratories. However, the NIST standard reference glasses that are currently available for instrument calibrations and quality control, such as NIST 612/4 and 1830/1, were not designed for forensic purposes. They were designed for chemical analysis of lunar material, such as the 61x series, or for major element analysis, 183x. Both types of analyses were performed on the bulk-scale, with hundreds of mg of material used for each analysis.<sup>2,3</sup> When these materials are used as calibration standards for analyses conducted on the micro-scale, there are potential heterogeneity biases that will inherently affect the precision and accuracy of subsequently reported data.<sup>4</sup>

This study's goal is to re-analyze widely used NIST glass SRMs so that their trace element abundances are reported on a scale that is useful and appropriate for micro-scale forensic glass analyses through a combination of multiple analytical techniques. The first will involve the use of nuclear analytical techniques, such as instrumental neutron activation analysis and prompt gamma activation analysis, to analyze the bulk glass materials and determine additional forensically useful elements.<sup>5,6</sup> The use of LA-ICP/MS will allow us to determine the degree of heterogeneity present on the glass wafers at the micro-scale.<sup>7</sup> The combination of these two techniques will allow for the modification of existing, and provide new, trace-element data that is appropriate for the sample size of forensic analysis. The methods developed during this work will ultimately be used to analyze and report on the trace element abundance of new float glass standards designed to supplement the existing float glass standards (FGS glasses), with the goal to certify the new material as a NIST SRM specifically for the forensic community.<sup>3</sup>

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#### Trace Elements, Instrumental Analysis, Glass





### D17 The Strange Case of the Magnet on the Electricity Meter: When Wrongly Executed Tests Show a Non-Existent Tampering Effect

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**Learning Overview:** After attending this presentation, attendees will better understand the problems that arise when wrongly collected evidence may result in people being unrightfully prosecuted for aggravated theft. Attendees will learn how to critically analyze all available data to assess whether a magnet laid on the top side of an electronic electricity meter can force the meter to measure less energy than that flowing through the metering section.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight on an incorrect practice for collecting evidence adopted by the Italian Distribution System Operators (DSOs) in an effort to counteract illegal attempts to steal electrical energy by placing electricity meters inside a strong magnetic field in the belief that this may alter the measurement error in favor of the consumer.

In Italy, the task of metering electrical energy at the delivering section for billing purposes is assigned exclusively to the DSOs. At the beginning of this century, they started to replace the old, electromechanical energy meters with more modern electronic meters, which can be read and controlled remotely and allow for implementing customized billing policies.

The very first model, installed before the transposition of the Directive 2004/22/EC of the European Parliament and of the Council on measuring instruments in the national law, exhibited a poor design of the current transducers whose magnetic core could be saturated by a strong DC magnetic field.<sup>1</sup> For this reason, unethical customers placed a strong magnet on the top side of the meter in the hope that this could reduce their energy bill.

After the Measuring Instruments (MID) directive was transposed in the national law in 2007, the DSOs started to replace the very first-generation meters with a new version that complied with the MID requirements.<sup>1</sup> Since the MID requires that in the presence of a magnetic field, the maximum permissible error is not exceeded by more than an additional 2%, the new meters are immune to the effects of external magnets.

However, some customers, generally the owners of small pubs, restaurants, and pizza parlors for whom the electricity cost represents a relevant share in the total costs of their business, still believe that magnets laid on the meters can force the meters to measure lower energy values and the DSO's verification teams still find such situations.

When this happens, the DSO makes a report to the judicial police and the consumer is prosecuted for aggravated theft and quantification of the amount of stolen energy, also determined by the DSO. However, in most cases, the quantification, based on verification tests performed only by the DSO technicians without providing the customer the right to have his/her own technical experts attending the test operations, are incorrect and a strict analysis of the available data, the historical data, and the test report shows that no additional measurement error can be attributed to the presence of the magnet.

This presentation will discuss how those data must be analyzed to state, in a scientifically and technically sound way, whether the electricity meter was tampered with or not and presents the result of some actual cases that led to release of the defendant for not having committed theft.

**Reference(s):**

<sup>1</sup> Directive 2004/22/EU of the European Parliament and of the Council of 31 March 2004 on measuring instruments (MID) (2004).

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**Tampering, Illegal Practice, Evidence Collection**



## D18 Qualifications for Professional Work in Forensic Linguistics

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**Learning Overview:** After attending this presentation, attendees will better understand the consensus in the field of forensic linguistics regarding what constitutes qualifications for professional work in forensic linguistics.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing guidelines for evaluating expertise in forensic linguistics and what can be expected of experts in forensic linguistics.

Linguistics is the discipline that employs scientific methodology to understand language.<sup>1-4</sup> Linguistics uses social science methods for data collection and analysis, statistics for quantitative analysis, and computer modeling. Linguistics is applied in current technologies such as internet search engines, voice-activated assistants, chatbots, and speech-to-text dictation services. Forensic linguistics is usually defined as the application of linguistics to forensic questions such as identifying authorship, assessing types of texts, and classifying communications.<sup>5,6</sup>

In the field of forensic linguistics, many non-linguists have presented themselves to the courts as experts in forensic linguistics. Because few judges have direct experience with linguistics as a scientific enterprise, such non-linguists have sometimes been able to convince courts that non-linguists should be considered experts on linguistic issues. Non-linguists who present themselves as “forensic linguists” often have degrees in computer science, English literature, foreign language literature, education, or rhetoric and technical writing, without any academic training in linguistics.<sup>7</sup> It is now possible for individuals without any training in linguistics to become “forensic linguists” by gaining graduate degrees in “forensic linguistics” (rather than linguistics), take a week-long course in forensic linguistics, or take a six-week online course, or join an organization of “forensic linguists.” These degree programs are controversial in the field because none of them offer strong academic programs in the core fields of linguistics.<sup>8</sup>

For actual linguists who are concerned about the quality and use of experts within the judicial system, the presentation of non-linguists as linguistic experts is troubling. The situation of non-linguists presenting themselves as forensic linguists and experts is becoming more likely as the value of linguistic evidence is more widely recognized. The community of linguists is now responding to this issue. This study presents the consensus in the field of linguistics related to qualifications for professional work in forensic linguistics.

There is consensus among leaders in the field of forensic linguistics that some benchmark qualifications are needed for professional work in forensic linguistics. The necessary and standard qualifications for performing forensic authorship identification are twofold.<sup>1,7-13</sup> First, the forensic linguist must have an earned doctorate in linguistics with demonstrated competence in subspecialties that relate directly to the forensic tasks in which the linguist works. Second, the forensic linguist must have some training or professional experience in forensic science methodology and legal standards for scientific evidence. These two benchmarks are attainable. Linguistics has many subspecialties that relate to forensic issues.<sup>14</sup> Further, there are many avenues through which linguists can obtain training in forensic science and legal standards. These benchmark qualifications ensure that the expert respects and follows legal standards for forensic linguistic evidence by producing “ordinary science” using standard analytical techniques from linguistics. If these benchmark qualifications are used to evaluate forensic linguists, the courts will make wise decisions about hearing forensic linguistic evidence because the evidence will be grounded in linguistic theory and methodology and will be prepared to meet legal standards of admissibility. In reality, only such evidence is useful.<sup>15,16</sup>

Forensic linguistic testimony can also come from non-academic experts, such as the police officers, digital forensic examiners, forensic document examiners, forensic psychologists, and medical examiners. It is not realistic to expect that these experts should spend five to seven years obtaining a doctorate in linguistics from a respectable program in order to meet the benchmark qualifications. Yet these experts certainly are appropriate and necessary consumers of forensic linguistics. For this reason, software has been developed that implements doctoral-level analytical methods. Such software is used by non-linguists to obtain valid results of linguistic analysis, with known error rates, data thresholds, and methodologies.<sup>17</sup> While such software cannot replace the linguist, such software does provide non-linguists access to valid and reliable forensic linguistics. Such experts are known as forensic linguistic technicians and forensic linguistic examiners, but not as forensic linguists.

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### Expert Witness Qualifications, Forensic Linguistics, Forensic Technicians and Examiners



## D19 A Validation Test of SynAID in Authorship Attribution Using Legal Transcriptions

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**Learning Overview:** After attending this presentation, attendees will better understand the feasibility of using one method of Syntax-Based Authorship Identification (SynAID) when part of the evidence involves spoken dialogue.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing quantitative evidence regarding the limitations, if any, of using well-vetted transcriptions of spoken dialogue for the purpose of syntax-based authorship attribution.

This presentation reports the results of a research project to evaluate the ability of a syntax-based method for authorship identification. The SynAID functionality in the ALIAS software program was used to identify the author of each of six samples of transcriptions of spoken responses during legal proceedings. Each of three individuals from a single speech community produced responses to a lengthy set of questions during a deposition; they then produced further spoken testimony at trial. All responses were transcribed by professional court reporters.

In order for an authorship attribution software system and protocol to be considered capable of identifying the author of a questioned document, it should be proved capable of correctly identifying the author of known documents.<sup>1-3</sup> A statistical model of the known documents can then be used to classify the questioned documents, as is the standard practice in classificatory statistics.<sup>4-6</sup>

This project considers six sets of statements spoken by three individuals. Every sentence in each set of statements is analyzed syntactically, and the part of speech (or syntactic function) of each word is coded. Once the coding is complete, Statistical Package for the Social Sciences' (SPSS's) Discriminant Function Analysis (DFA), following SynAID protocols, is used to construct a model of the speaker's typical syntactic structures.<sup>2,3</sup> In this case, two models for each speaker will be created, one from the deposition and the other from court testimony.

The validation testing for the authorship attribution of transcribed speech will be considered successful if, based solely on the speaker's syntactic patterns and without regard to the content of the speaker's utterances, the deposition testimony of Speaker A is identified as having the same author as the court testimony of Speaker A, and so on for the other two individuals. Further, the validation testing will be considered successful if Speaker A is differentiated from Speaker B, and so on for the other two individuals. Since the material is dialogic, this study also measured the amount of overlap between the interlocutors as an indicator of mutual understanding as well as potential syntactic interference.<sup>7</sup>

Validation testing has been previously performed on SynAID for written documents in cross-genre documents, such as business letters, love letters, apologies, trauma narratives, and essays.<sup>1-3</sup> Further, validation testing has been previously performed on SynAID for text messaging, where all documents were within the one genre of texting, with the texts bundled into chronologically sequential units of 100 texts.<sup>8</sup> This is the first validation testing that involves spoken language rather than written language. SynAID has not previously been tested using transcribed speech samples. This project will thus provide evidence that SynAID is or is not an appropriate tool for authorship attribution using transcriptions of spoken dialogues.

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Authorship Attribution, Quantitative Evidence, Forensic Linguistics

### D20 Challenges and Opportunities for Integrating Epistemic and Evidential Information Into Author Recognition Systems

E. Allyn Smith, PhD\*, University of Quebec at Montreal, Montreal, PQ H3C 3P8, CANADA

**Learning Overview:** The goal of this presentation is to introduce epistemic modality and linguistic evidentiality to the broader forensic science community, presenting empirical results with respect to the potential fitness of these variables for computational linguistic systems. Attendees should be able to understand and evaluate the relevance of these variables for, as an example, authorship analysis systems.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting theoretical and empirical results related to the integration of semantico-pragmatic variables into existing author recognition systems, putting them in the hands of those who are best positioned to apply them to state-of-the-art computational linguistic systems.

Most successful author recognition systems, whether computational linguistic systems (to be preferred in legal applications) or computational stylometric systems, rely more heavily or even exclusively on morphological and syntactic linguistic variables (including here characters and n-grams), partially because these lend themselves more readily to quantification and computational systems.<sup>1-3</sup> Semantics, roughly the study of the literal meaning of utterances, and pragmatics, the study of meaning in context, are less often present (though mentions incorporating discourse functions, and there is recent interest in moving in this direction).<sup>4,5</sup> Systems sometimes mention semantic information as being important, but this often boils down to word choice, such as identifying low-frequency words occurring at a statistically higher-than-normal frequency in a corpus of texts by a given author. In this case, it is the mere presence of a word and not its meaning as such that is of interest. It is not surprising that meaning tends to be neglected, as it is seen as more ephemeral and, indeed, can be hard to pin down despite being related to the syntax. Many aspects of semantics and pragmatics are also known to be genre-specific, which poses an additional challenge for systems that strive for general application.

The two main steps in true computational linguistic authorship systems are the extraction of variables representing the author's style, and the application of various algorithms to the variables in the first step for the purpose of modeling.<sup>1,6</sup> As explained, efficient variables for these systems will have two features: low within-author variability, and high between-author variability.<sup>4,6</sup> This presentation addresses the variable-extraction step, offering an academic linguist's take on semantic and pragmatic variables that might fit the bill of being variable across authors/speakers and less variable within a given author/speaker.

The variables considered here are epistemic modals and markers of evidentiality. Epistemic modals are words and phrases that speakers use to communicate their degree of certainty or commitment to their utterances (or lack thereof).<sup>7</sup> In English, these might include "I'm sure that," "apparently," "I don't know," or even "ummm" or "well," among many others. These are sometimes also called "hedges" in other literatures, especially when expressing uncertainty. Evidential markers give the basis for a speaker's claim.<sup>8</sup> These include expressions such as "I heard through the grapevine," "I saw," etc. Evidentials can implicate epistemic meanings as well. For example, speakers may choose to specify that their statements are hearsay in order to distance themselves from the information.

In this talk, Empirical results from certain works and previous studies that delve into the application of epistemic modals and evidentials in legal settings will be presented.<sup>9,10</sup> Building on earlier work distinguishing legitimate 911 emergency calls from false calls, corpus studies show that legitimate calls contain statistically more epistemic modals than false calls, including those in which a killer calls and feigns innocence, presumably because real witnesses want to be sure that they do not overstate information they are unsure of in a high-stakes situation.<sup>10,11</sup> Speakers often choose to repeat the subtype of epistemic modals they use, which is a further advantage as it distinguishes them from one another. This study tests whether an authorship identification system could define a group of epistemic modal and evidential phrases and search for their relative usage rather than just searching for the presence/absence of one of the many of them to see whether this variable cluster would correctly distinguish authors.

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#### Computational Linguistics, Semantics, Pragmatics

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### D21 Detecting Linguistic Markers of Religious Extremism in an Online Environment: A Pakistan Case Study

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**Learning Overview:** After attending this presentation, attendees will better understand the role of the internet, social media, and sermons in radicalization of youth in Pakistan, and of forensic computational linguistic analysis for detecting linguistic markers of religious extremism.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing multilingual English-Arabic-Urdu text analysis for detecting linguistic markers of extremism for proactive investigations.

The phenomenon of “extremism,” “religious terrorism,” or “violent radicalization” has grown in recent years, associated with several ideologies. Pakistan is among the top five on the list of countries affected by terrorism/violent extremism.<sup>1</sup> The war on terrorism has killed nearly five million people in Pakistan, Afghanistan, and Iraq combined, resulting in displacement of families, as well as the clear emotional and mental issues arising from such turmoil.

Social media has been playing a vital role in propagating extremism through radicalization of youth. The advantages of using the internet and social media include communication channels that are not bound to national jurisdictions and that are informal, large groups, cheap, decentralized, and anonymous.<sup>2,3</sup> These channels are used in several ways to attract a young audience, publish violent content based mostly on false information, and persuade/recruit youth for violent acts of terrorism. It has become difficult to differentiate between a religious website that publishes only for the sake of awareness/knowledge and pages/websites where exaggerated/made-up religious stories are posted for a sinister agenda. The current pilot focuses on Pakistan as it is among the top ten internet users in Asia with manifest online problems.<sup>4</sup>

Specialized software can play an important part in identifying such platforms and tracking down terrorist organizations by *detecting warning signals/threats* associated with such activities. Machine learning techniques can be used effectively to detect “weak signals,” “digital traces” of “linguistic markers” that characterize the warning signals/threats associated with terrorism or religious extremism. The warning behaviors that have the highest potential to be discovered in text and speech content are leakage (the communication of intent to do harm to a third party), fixation (increasing perseveration on the object of fixation), and identification (a desire to identify oneself with previous attackers or a terrorist organization).<sup>5-9</sup> Previous examinations of warning signals have been developed from forensic psychology and behavioral sciences, sociology, and computer science.<sup>3,6,7,9,12,17</sup> Based on previous studies, a definition of “extremist ideology” as an invocation to violence against specific groups justified by an ideological position (e.g., religious affiliation, racial superiority, and other extremist ideas) is used. “Extremist language” is then defined as the invocation through language.

The examinations that actually consider language, however, focus on content analysis, sentiment analysis, critical discourse analysis, and basic corpus linguistics.<sup>14-16,18</sup> Rigorous theoretical linguistics has generally been missing from the discussion.

This presentation focuses on developing objective, operational definitions of “leakage,” “fixation,” and “identification” from the perspective of formal linguistics and computational linguistics. Formal linguistics includes both semantic and syntactic theory, and computational linguistics uses algorithms for syntactic, semantic, discursive, and orthographic textual analysis. These methods and techniques can be used for any kind of language-based analysis, but this pilot study focuses specifically on data related to Pakistani radicalization. The pilot for methodological development focuses on detecting the warning signals/threats through linguistic markers of religious extremism in an online environment, with linguistic markers defined from linguistic theory and computational algorithms. Data are collected from social networks (Facebook®, Twitter®), websites publishing religious content, which have more than 10,000 followers/subscribers in active discussions.

Data are analyzed using standard methods in linguistics implemented in the Automated Linguistic Identification & Assessment System (ALIAS).<sup>10,11</sup> First, in line with previous studies, ALIAS is used to calculate quantitative rates for words related and unrelated to extremist ideology. This helps to determine which key words or phrases show up repeatedly and provides a baseline of expected terminology of extremism in the social environment. Second, ALIAS is used to perform syntactic and semantic analysis of passages related and unrelated to extremist ideology to determine if there is a correlation between the content (extremist thoughts/ideas/messages) and syntax (what’s the syntax in extremist vs. non-extremist phrases/sentences). The methodologies based in linguistics offer another tool for identifying radicalization through language, focusing on both salient and sophisticated features of language.

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### Religious Extremism, Linguistic Markers, Religious Extremism in Pakistan



### D22 Forensic Linguistic Research Collaboration Between an Industry Research Institute and a Forensic Science Laboratory

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**Learning Overview:** After attending this presentation, attendees will better understand the challenges and feasibility of conducting research in a collaboration between an industry research institute and a forensic science laboratory.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how data can be collected, stored, and managed within the forensic science laboratory for research purposes beyond casework.

Without dispute, the forensic science laboratory's main purpose is investigation of crime science evidence. Laboratories are funded to provide this service to the criminal justice system. Another, less-developed but no less important, function of the forensic science laboratory is research. It is difficult to conduct both investigation and research within the forensic science laboratory because investigation always takes precedence over research. Additionally, investigative analysis must be completed in short amounts of time (e.g., days or weeks), while research projects can extend over longer periods of time (e.g., years). The urgent request demands immediate attention; time allotted to the research project must be allocated between urgent requests.

The disconnection between the forensic sciences and research science is well documented.<sup>1</sup> It has been argued that this disconnection is the cause of failures within forensic science.<sup>2</sup> Therefore, the connection between forensic science and research science must be developed so that forensic science advances and the culture of research science pervades forensic science.<sup>3,4</sup>

This presentation details how a collaboration between the Institute for Linguistic Evidence in the United States of America and the Forensic Science Centre in Croatia is conducting research in forensic linguistics. At the Centre, anonymous letters in Croatian have been collected in relation to crime scene investigation. These anonymous letters and known exemplars from suspects have been used in forensic handwriting examination and other types of investigative methods. Such data can also be used for research purposes.

In order to convert data from its investigative purpose to a research purpose, the forensic laboratory must consider three issues: storage, management of access, and experimental designs.

**Storage:** For casework, the obvious storage of items is within the case folder. Each case has its own folder, and so linguistic data is distributed over the case management system in folders where the case happened to involve linguistic evidence. For research, the items must be stored in a central repository that is designed to hold, identify, and use the items. Exact copies of the linguistic evidence in the case folders must be replicated for storage in a central repository.

**Management of Access:** For casework, the obvious management of access must follow chain of custody and peer-review standards. The investigative items are tracked and tagged within an evidence system. The people who have access to the data are employees of the forensic laboratory. Access to the investigative items is restricted to the forensic scientists conducting the examination and any supervisors who may be conducting peer review of the examination. For research, it is important that researchers who access the linguistic evidence also follow chain of custody. The access must be enlarged because some researchers may not be employees of the forensic laboratory. For instance, researchers may be statisticians, software developers, and linguists who work with the forensic scientists. These researchers must comply with the forensic science laboratory's standards for handling and encrypting data; they cannot remove data from the central repository, and they cannot give access to the central repository to others who are outside the research team. Confidentiality and privacy are shared values in both casework and research.

**Experimental Designs:** For casework, the experimental design is determined by the facts of the case. For research, the purpose of the research can drive the development of experiments beyond the original question posed by the facts of the case. For example, in casework the issue may be authorship, but additional experimental designs may allow the data to be used for threat assessment, suicide note assessment, predatory chat, and other issues in forensic linguistics.

Research within the forensic science laboratory is feasible. It takes time and effort to select the research partner. Such collaboration is a feasible way for forensic science to connect with research search, while maintaining its primary purpose.

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#### Forensic Science Research, Forensic Linguistics, Forensic Science Laboratory



### D23 Formalizing Spanish Markedness: Working Toward a Spanish Version of the Automated Linguistic Identification & Assessment System (ALIAS) Syntax-Based Authorship Identification (SynAID)

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**Learning Overview:** After attending this presentation, attendees will better understand the motivation, challenges, and feasibility of developing a syntax-based method for Spanish authorship identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enabling multilingual English-Spanish authorship identification for criminal investigations.

The Institute for Linguistic Evidence (ILE) has embraced the forensic computational linguistic paradigm from its inception in 1998, confronting the tradition of academicians functioning as expert witnesses in forensic linguistics without sustaining a proper research agenda in the field or without testing their methods for error rate. An ILE team performs scientific analysis firmly grounded on linguistic theory, in line with Labovian linguistics that “validity and utility are not distinct, [...] theory only has value to the extent that it is relevant to real-life problems.”<sup>1</sup> Thus, ILE researchers develop and/or adopt scientific methods that have been devised and tested for accuracy outside of any litigation, since the ILE paradigm attempts to promote a real forensic science that is useful to the judicial system, involving reliable, replicable, and respectable research.<sup>2</sup>

Within this framework, research conducted at ILE is grounded on research questions arising from the observation of practical problems in the forensic setting.<sup>3</sup> ILE researchers make use of computational linguistic analysis software, specifically ALIAS, as well as statistical software, such as SPSS, DTREG, and statistical analysis algorithms in Python.<sup>3-6</sup>

The main current language in ALIAS is English, and Spanish is among the research-in-progress languages. Accordingly, the present proposal puts forward the ongoing project aiming to develop the Spanish version of this computational tool. In particular, research and development has focused primarily on developing a Spanish version of SynAID.

Specifically, SynAID applies standard techniques of syntactic analysis from the core field of linguistics known as syntax.<sup>7-9</sup> Syntax is the mental process of combining words into phrases and phrases into sentences, so that meaning can be conveyed through language. Syntactic analysis is the process for identifying, within a sentence or phrase, each word’s syntactic relationship to the other words with which it is being combined. In the sentence, “The dog bit the mailman,” the words *the* and *dog* combine to make the phrase *the dog*, a noun phrase; the words *bit*, *the*, and *mailman* combine to make the phrase *the mailman*, which itself combines with *bit* to make the verb phrase *bit the mailman*. and *bit the mailman* combines with *the dog* to make the sentence *the dog bit the mailman*. Although syntax is unconscious and automatic, it has been proven to be psychologically and neurologically real.<sup>10,11</sup> Further, the memory of syntax degrades in milliseconds, as we focus on the meaning rather than the form.<sup>12</sup> Syntax is a core field within linguistics and is accepted within all paradigms of linguistics as central to human language.<sup>7-13</sup>

Further, the SynAID method for authorship identification is based on the advanced analytical method known as syntactic markedness, that is to say, the complexity of phrase structures, materialized in linguistic phenomena, such as extrapositions, dislocations, inversions, and preposings and internal structures of phrases.<sup>7,8,14</sup>

For the syntactic design of structures in Spanish, every syntactic head was listed with all the possible patterns for that head; then, these syntactic patterns were subcategorized into marked and unmarked patterns by considering levels of complexity and other factors in markedness theory.<sup>14</sup> A considerable number of similarities were found across languages (English-Spanish); nonetheless, certain linguistic phenomena unique to Spanish deserved special attention, such as subjunctive mood and clitics.

As for the data collection process, data was elicited using an instrument designed for forensic linguistic evidence.<sup>15</sup> The questionnaire consisted of ten questions, in response to which participants authored a different type of text in Spanish. An Institutional Review Board approval was processed in order to protect the welfare, rights, and privacy of human subjects. Once collected, the data were not altered in any way, which led to protocols for handling severely degraded data. Furthermore, researchers complied with all the ethical requirements for forensic research, namely anonymity, confidentiality, preservation of the data, and restricted access to the data, as presented elsewhere.<sup>16</sup>

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### Forensic Computational Linguistics, Syntax, Linguistic Markedness



### D24 Driver Seat and Fuel System Failure in a Rear-End Collision

*Kurt D. Weiss, MS\*, Case Study Collision Science, LLC, Santa Barbara, CA 93190; Mark C. Pozzi, MS\*, Sandia Safety Sciences, Edgewood, NM 87015*

**Learning Overview:** The goal of this presentation is to share with the forensic science community the detailed methodology used to reconstruct a two-vehicle rear collision and to demonstrate the tear-down and examination of a Sport Utility Vehicle (SUV) seat assembly and fuel system that failed. Occupant kinematics analysis and alternative designs to prevent the failures and injuries will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the failure mode of a common SUV seat structure that rendered the lap/shoulder belt ineffective, and multiple fuel tank failures that resulted in the predictable catastrophic injury of a driver in a moderate rear-end collision. Loss of survival space of rear seat occupants, especially children, is also demonstrated.

**Collision Overview:** A stationary 2004 Jeep® Wrangler® was struck from behind by a 2005 Ford® F150 and propelled forward approximately 100 feet into a pole. As a result, the Jeep® spewed gasoline from its ruptured, fully crushed fuel tank and the driver seat back collapsed and rotated rearward, allowing rearward driver ejection and forceful head contact into the rear compartment. The driver's head strike resulted in cervical spinal trauma and permanent paralysis.

**Physical Evidence:** Vehicle examination showed an offset distance of approximately 21 inches between the Jeep® and F150 centerlines at impact. The Jeep® sustained an average rear crush of approximately seven inches. The Jeep® frontal airbags did not deploy. The Jeep® driver's shoulder belt, found wrapped around his lower legs, caused police to misinterpret belt use and placement.

**Reconstruction:** The vehicle crush profile, weights, and stiffness coefficient analysis demonstrated a F150 impact speed of approximately 41mph and Jeep® velocity change of approximately 26mph with a Principle Direction Of Force (PDOF) of 6 o'clock during the rear impact and approximately 15mph impact of its right front corner into the pole.

**Injury and Occupant Kinematic Analysis:** In the rear impact, the properly lap-shoulder belted driver slid rearward relative to his vehicle interior, ramped up his collapsing seat back, struck his head in the rear compartment, and was partially ejected through the rear window. When this occurred, his safety belt was no longer in contact with the body it was designed to protect. The safety belt pass-through latch plate allowed webbing transfer from the shoulder belt to the lap belt, contributing to the ineffectiveness of the safety belt. The driver's catastrophic neck injury and paralysis was the direct result of the identified seat failures.

**Vehicle Defects:** Fuel system and seat defects were identified. The aft-of-axle fuel tank breach and filler neck separation with no check valve resulted in catastrophic fuel leakage. Spilled fuel failed to ignite into what most likely would have been a fatal fire. The fuel system in the rear crush zone was the same as the 1960's design. The driver's seat revealed predictable catastrophic buckling of seat recliner mechanisms at large lightening holes and adjacent weak structures. Static testing of the Original Equipment Manufacturer (OEM) and modified OEM seats replicated this failure mode and demonstrated inadequate seat strength to absorb predictable occupant loads in moderate rear collisions.

**Alternative Designs:** Alternative fuel tank and seat designs were identified and shown to be capable of preventing rearward occupant ejection and catastrophic injury. These designs were available and technologically and economically feasible at the time the vehicle was designed and produced; in fact, these alternate designs were installed by the same automaker in other model vehicles. Absent these defects, the driver seat back would not have rotated rearward, his safety belt would have effectively coupled him to his seat, he would not have ramped up his seatback, there would have been no head contact with the rear occupant compartment, and, therefore, there would not have been injurious neck loading and residual paralysis. Implementation of alternative designs would have prevented the observed failures and injuries.

**Conclusion:** In this crash, the Jeep® demonstrated foreseeable failures of the fuel system and weak driver seat structure resulting in catastrophic injury. Implementation of available, and technologically and economically feasible, alternative designs would have prevented these failures and resulting injury. Principles of crashworthy seats, fuel system design, and preservation of survival space were known to automakers and the Department Of Transportation (DOT) since mid-1960s.

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#### Seat Failure, Fuel System Failure, Rear-End Collision



### D25 Electrical Bike (E-Bike) Deceleration Analysis Using Advanced Electronic Systems

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**Learning Overview:** After attending this presentation, attendees will be more familiar with E-bikes, the operating system of the tested Greyp bike, and possible ways of E-bike examination in digital forensics and road accident analysis areas.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about smart E-bikes, as well as the road accident examiners in their daily work with similar cases.

The goal of this presentation is to explain forensic acquisition of E-bike memory in closed and factory-created systems (such as embedded Linux in Greyp E-bike), as well as the memory analysis using commercial and open source forensic software.

In the area of road accident analysis, deceleration of a vehicle is one of the most important factors to calculate initial speed of the vehicle before a crash. Providing braking tests, this study was able to establish the minimum stopping distance of a vehicle in testing conditions and by using such data, calculate the vehicle deceleration. Yet, testing conditions could be slightly different in comparison with the real road conditions. While the asphalt surface in a test area is usually new and rough, real road asphalt surfaces are often totally different (usually smooth with some cracks and other types of damage, covered with dust or mud).

In the Forensic Science Centre “Ivan Vucetic,” two different methods were used for determining braking distance of a vehicle: static measurement using the ASFT t2Go device and dynamic measuring using the XL meter. Using both methods separately or together, this study could establish or calculate deceleration of a vehicle.

The idea of using data from the Engine Control Unit (ECU) of E-bike Greyp came up as a logical follow-up in this area. By reading the data from the bike ECU, it is possible to get information such as the bike speed, acceleration/deceleration, time and way of braking, route of driving (using Google® Maps or a similar app), and many other data. Data given from the Greyp is automatically stored in the operating system of the bicycle and on the storage server. Some data also gets stored on the user’s mobile phone device paired with the bicycle, making it accessible to the user for viewing through the mobile user application.

This presentation will show the process of the Greyp E-bike physical memory acquisition and Read-Only Memory (ROM) structure of the extended file system (ext). Forensic examination and memory analysis will be conducted with the commercial and open source software. Information obtained from the Greyp ECU and from the Greyp operating system will be compared with the data collected and analyzed by the usual forensic methods after conducting vehicle dynamic tests of the given bicycle.

Using digital forensic techniques (acquisition and extraction) and methods (parsing and analysis), this study can confirm (or deny) results of a road accident expertise and maybe discover new data relevant to similar cases.

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#### Road Accident Expertise, E-Bikes, Braking Distance



### D26 Rear Seat Crashworthiness: Predictable Failures of Seats, Belts, Liftgates, Vehicle Structures, and Loss of Occupant Survival Space in Rear Impacts, Especially in the Third Row

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**Learning Overview:** This goal of this presentation is to show rear impact case studies and tests involving forensic investigation of catastrophic ejection, injuries, and fatalities due to failures of vehicle second and third row seating area structures, seats, and restraints. Parents are advised to place children in such seats to avoid airbags, but no tests are performed by automakers to determine actual occupant protection in rear impacts. No rear impact occupant protection standards exist. Flawed and inadequate seat standards allow predictable failures of structures, doors, seats, and belts in low-speed rear crashes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how and why restrained adult and child occupants are predictably ejected, injured, and killed in low-speed rear crashes. Hidden dangers of trailer hitches, weak seat structures, slackened belts, weak liftgate, and door latches are depicted. The lack of testing by automakers or the Department Of Transportation (DOT) to evaluate rear impact occupant protection, especially in rear seats, will be explored in detail. Investigators of such collisions will be better able to evaluate injuries and forensic evidence related to rear seat occupants.

Second and third row rear seats in vehicles receive little to no attention during design, development, or testing because loopholes in United States safety regulations and automaker internal standards do not require meaningful performance evaluation of rear structures, restraints, seats, cargo shift/rear intrusion, or occupant protection. Significant improvement has occurred since 1979 in vehicle frontal and side crashworthiness due to the competitive challenges posed to automakers by the National Highway Traffic Safety Administration (NHTSA) New Car Assessment Program, including front, side, and rollover airbags; adjustable seat belt anchors; pretensioned seat belts, and improved side structures. However, there has been little-to-no corresponding improvements in rear seat crashworthiness and occupant protection. Many vehicles continue to operate with rear lap-only belts, no head restraints, weak seatback structures combined with pass-through bulkheads from rear cargo areas; seat cushions with poor anti-submarining structure, no cargo tie-downs, netting, or shields, and other significant flaws that significantly increase risks to rear seat occupants. In 1996, the DOT admitted that their seat safety standard was “flawed and inadequate.” That same year, automakers and the DOT issued warnings that children should be placed in the second or third row to avoid airbags. The DOT and automakers continue to follow the “flawed and inadequate” seat safety standard. Not surprisingly, due to these flaws, independent crash researchers discovered children are 61% more likely to incur severe or fatal trauma in rear crashes than in any other impact vector.

Case studies, testing, and simulations that illustrate these predictable dangers are analyzed in detail. As demonstrated in numerous investigations, automakers have admitted that as “normal practice,” they never measure, or even evaluate, second or third row designated seating positions in rear crash tests. For example, one automaker conducted more than 15,000 frontal sled tests and only 8 rear tests. The lack of testing, regulation, and automaker interest in rear seat occupant protection has led to significant trauma and death in readily survivable crashes. Predictable loss of rear occupant survival space due to front seat failure and belt slackening, as well as cargo shift and rear intrusion, has caused rear seat occupants to be ejected into rear vehicle structures or out of the vehicle, rammed into the roof, and inadequately restrained by belts slackened due to seatback and seat track failure.

Trailer hitch receivers have been added to rear vehicle structures without any testing to determine what effects those hitch receivers would have on rear impact crashworthiness and occupant protection. This has caused rear “crush zones” to be completely altered and rendered ineffective. This has not only decreased the energy absorption capabilities of vehicles but also translated crush far forward into the occupant compartment, causing loss of survival space. This also increased the severity of crash forces affecting vehicle occupants, with corresponding increased loads on occupants, seats, and belts.

Front and rear crash tests and static tests have been conducted and have demonstrated these predictable hazards, as well as safer alternative designs that are readily available, technically feasible, and cost effective.

#### Rear Impact, Rear Seat Safety, Crashworthiness



### D27 Sliding Distance Measurements and Their Role in Pedestrian vs. Vehicle Accidents

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**Learning Overview:** The goal of this presentation is to provide educational information concerning pedestrian accident reconstruction and the impact of documenting the physical evidence because analysis using less accurate measurements may change the cause of the collision and therefore affect potential liability of the party.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the importance of physical evidence measurements and documentations in the liability process.

Pedestrian position relative to the vehicle determination in accident analysis often is an important factor to find the true cause of the accident and the liability process. In many accident cases, small derivations in primary parameters would affect the results and, thereby, the liable party dramatically. In many situations, the only elements remaining at the venue are final positions of the vehicle (if the vehicle has not been relocated prior to police officers arrival) and the pedestrian after the crash. One important element that is often not documented at the scene is the sliding distance of the pedestrian. Evaluating the remaining evidence, which could lead to measuring the sliding distance, can define the location of the pedestrian at the time of impact with the vehicle. Hence, often based on those elements, an adequate model can be utilized to reconstruct the accident and provide conclusions about the crash sequences.

There are multiple equations developed to investigate and analyze vehicle-pedestrian impacts. The majority of these calculations are focused on the total throw distance and the vehicle speed at the time of the impact. While those parameters play a significant role in reconstructing the accident scene, often the input parameters to properly calculate those values are not adequately available. One methodology that can often help to locate the position of the vehicle and the pedestrian relative to the roadways and relative to each other is measuring the sliding distance. Oftentimes, crucial measurements of the physical evidence that may provide a more accurate sliding distance are not documented by the investigator. By knowing the geometry characteristics of the vehicle and the pedestrian, and by applying the sliding distance, the launching velocity of the pedestrian can be calculated. This presentation uses a pedestrian vs. vehicle traffic fatality to explore the analysis using slide distance to calculate the total throw distance. Based on the sliding distance in the police report and using the road surface coefficient of friction measured at the site by the expert, the pedestrian's launch velocity was more accurately calculated. Subsequently, the vehicle impact velocity at the time of separation was calculated. As a result, by applying the vehicle range of speed at the time of impact and other parameters, such as the pedestrian launching height and launching angle, the total throw distance can be calculated. Since in many cases it is difficult to measure the total throw distance due to lack of physical evidence and locating the initial impact area relative to the roadways, calculating the total throw distance can play a significant role in identifying the liable party and who was at fault causing the accident.

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#### Forensic, Accident Reconstruction, Pedestrian vs. Vehicle Collisions



### D28 Face It: A Dangerous Passing Maneuver in an Elite Cycling Event Can Have Fatal Consequences

*Billy S. Cox, Jr. \*, Billy Cox Group, Navasota, TX 77868*

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**Learning Overview:** The goal of this presentation is to determine the impact speed and forces involved in fatal bicycle crash using GoPro® video and testing of materials.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the techniques utilized to reconstruct a bicycle crash that occurred during the 2016 Tour of Kansas City (KC). During the race, a crash occurred, resulting in the death of one of the cyclists. The performance of the fencing material used to separate spectators from the racecourse at the Tour of KC cycling race was at issue. Tests were conducted to determine whether fastening the fence panels together with zip ties would have minimized the injuries or prevented the fatal injury that the rider sustained in the collision.

The collision between two cyclists occurred when the fatally injured race competitor attempted to overtake and pass another cyclist on the left side between fatally injured rider's position and the barricades that separated the spectators from the racecourse. The cyclists became entangled and crashed into a spectator barricade. The rider that was overtaken was wearing a GoPro® camera on his helmet. His GoPro® video captured the event.

Upon being struck by the co-joined cyclists, the subject barricade began to move laterally separated from the next barricade in the sequence. The fatally injured cyclist was ejected over the bicycle handlebars. He struck the end of the next barricade panel, exposed due to the lateral crash forces exerted on the panel, with the center of his forehead, just below his bicycle helmet.

The rider that was being overtaken wrote in an email, "I did look at my footage from previous laps and the barriers weren't separated that far as I show on the picture. I believe when he (the fatally injured rider) first made contact with the fence it pushed that panel back, exposing the corner of the next panel. I do think zip ties would fix this issue going forward."

A test protocol was developed and a series of tests were performed to determine the approximate weight of a single barricade, the force required to move an unrestrained single barricade along a paved surface, and the force required to break the zip ties provided on a set of six barricades fastened together in a line. A series of tests was also performed to determine the force required to break the zip ties when they are pulled apart.

When the barricades were aligned in sequence and fastened with a single zip tie, the pulling strap was moved upward toward the top of the barricade (approximately 42 inches above the ground). The zip tie joining the subject barricade and the next in sequence broke when the peak force reached 55.25lbs. The pulling strap was moved upward toward the middle of the barricade (approximately 26.5 inches above the ground). The zip tie joining the subject barricade and the next in sequence broke when the peak force reached 77.79lbs.

The handlebar stem of the exemplar bicycle is about 38 inches above the ground, which is about 8 inches below the top of the barricade. The top of the seat is about 40 inches above the ground, which is about 6 inches below the top of the barricade. A cyclist would tend to load the top of the barricade in a collision wherein the loading force required to break the zip tie was 55.35lbs.

The subject barricade and the barricade which was struck by the fatally injured rider were correctly placed and aligned before the collision occurred. The subject barricade was moved laterally, exposing the next barricade in sequence that the fatally injured rider struck only after subject barricade was struck by the co-joined cyclists. The testing conclusively showed that the use of zip ties to fasten the subject barricade and the next barricade in sequence that the fatally injured rider struck would not have prevented the fatal injury.

This presentation will be highlighted with the GoPro® video, video from the barricade test, and reconstruction of the collision event.

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#### Crash, Bicycle, Reconstruction

### D29 Who Was Texting During the Alleged Kidnapping and Rape?

Carole E. Chaski, PhD\*, ALIAS Technology, LLC, Georgetown, DE 19947

**Learning Overview:** After attending this presentation, attendees will better understand how quantitative analysis of language use can identify authorship of texts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility of quantitative linguistic analysis for solving crime.

A woman and her estranged husband agreed to meet to discuss whether they were going to divorce or remain married. Since the husband was a long-distance truck driver and the wife stayed at home taking care of the children and horses, the husband and wife regularly texted each other throughout the day, even while they were separated. At one point they agreed to meet at his rig to discuss the state of their marriage. Both sides agreed to the meeting. Both sides even agreed that during the meeting the husband and wife had sexual intercourse. But the wife claimed that her estranged husband kidnapped her, because he would not allow her to leave, and raped her. The husband claimed that he had never kidnapped her and that he had not raped her because their sexual intercourse was consensual. The husband also said that after they had had sexual intercourse and were discussing their marriage, they argued and hit each other. The wife contacted the police; the husband was arrested and charged with kidnapping and rape.

During the investigation, an issue arose, from the defense's perspective, about the timing of the events. The beginning of the meeting was verifiable from text messages; the end of the meeting was verifiable from the police record. But during that time period, when exactly had the kidnapping and rape occurred? The wife's interviews showed that she had changed her mind about the timing of the events. Meanwhile, when the timeframe was finally decided upon by the prosecution and the defense, the text messages showed that there were 24 text messages sent from the wife's phone during that three-hour period. The defense wanted to know: who was texting from the wife's phone during the alleged kidnapping and rape? The wife claimed that her husband had taken the phone from her and was sending texts to her mother, grandmother, boyfriend, and son so that they would not worry about her. The defense, however, claimed that if she had access to her phone and was actually texting her family, she could have texted 911. The linguistic issue then became: who was texting?

Forensic authorship identification applies standard linguistics to the issue.<sup>1-4</sup> In this case, two methods for determining authorship were used. The first method is Syntax-Based Authorship Identification (SynAID).<sup>5,6</sup> The second method is keyboard dynamics, or typing patterns.<sup>5</sup> The data for both of these methods were more than 3,000 known texts sent between the husband and wife. From these known texts, a statistical model of the syntactic patterns of the husband and wife were developed. This statistical model of the possible authors' known writing had 96% cross-validated accuracy. Given the high level of accuracy for the known writing, the model of authorship was applied to the questioned items.<sup>7,8</sup> Of the 24 questioned text messages, 23 were assigned to the wife, with high typicality probability, and 1 was assigned to the husband. With an error rate of 4%, the one assigned to the husband could simply be statistical error, but even if the potential error were taken from the wife's assignments, for the benefit of the doubt, the wife's assignments were such that she would have had ample time to contact 911 or to ask her family to contact 911. The second method focused on the typing patterns since the main issue was who was actually typing on the phone. The husband and wife differed in their typing patterns; in particular, a differentiator was whether the text started with a space or a character. This pattern was favored by the wife rather than the husband. In the questioned texts, more texts started with a space than a character; this result converged with the syntactic method.

The prosecutor dropped the charges of kidnapping and rape. Because the husband admitted to hitting his wife during their argument after sexual intercourse, he was charged and convicted of other crimes.

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#### Forensic Authorship Identification, Syntax, Keyboard Dynamics





### D30 Electrical Fire or Arson Crime?

*Helmut G. Brosz, BASc\*, Forensic Science International Group, Markham, ON L3P 3P2, CANADA*

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**Learning Overview:** After attending this presentation, attendees will recognize that forensic electrical and metallurgical engineers can play an important role in analyzing a suspected arson scene. Electrical and metallurgical engineers can assist criminalists in determining if a fire could have been a criminal act, accidental, or unintentional. The manual sifting through screens of fire debris can yield valuable evidence from fire scenes when combined with metallurgical and electrical tests.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to an improved performance of criminalists and others involved in an investigation of a suspected criminal arson fire by availing themselves of forensic electrical and metallurgical engineering expertise.

This case study covers a fire in a manufacturing facility that puzzled a number of fire investigators. Some investigators opined the cause as arson, others opined the fire as accidental not involving human intervention, and others could not conclude a cause with a sufficient degree of engineering certainty.

The origin of the fire was agreed to be at a work station inside the factory floor open area that consisted of a wooden desk, computer on the floor, monitor, a 500VA Uninterruptable Power Supply (UPS) on the floor, extension cords on the floor, plastic waste basket, and a metal filing cabinet adjacent to flammable, cloth-covered metal screen walls close to the side of the building and next to another work station.

The fire occurred during the middle of a Saturday night when the plant was not in production. A worker had worked at the subject workstation earlier on Saturday morning. No accelerants were found in the vicinity of the workstation. No sign of a break-in or unauthorized entry was found.

Fortunately, all fire debris on the floor was swept up and kept in bags by the initial insurance investigators. cursory examination by investigators found no evidence in the debris that would point to a cause for the fire.

Detailed sifting of the fire debris from the floor, which was stored in bags, through fine screens produced important artifact evidence. Missing brass bus bar pieces and wiring from the 120-volt UPS were found. These artifacts allowed one to reconstruct the electrical bus bar system of this UPS that was heavily damaged by fire. One of the bus bars showed that the brass had been consumed/melted at a solder joint. Other adjacent solder joint locations **did not** exhibit such consumption/melting.

Metallurgical examination revealed that the brass adjacent to the missing brass reached temperatures in excess of 900°C (the melting point of brass). The plastic case material of the UPS was flammable and would ignite at this temperature. A new identical UPS was obtained, disassembled, and the brass bus bar system and its solder joints were compared. Electrical tests and research revealed that low current (less than one ampere of current) can cause overheating and arcing sufficient to initiate a fire under the right circumstances within a flammable material enclosure.

The conclusions reached were that the defective solder joint arced, consumed the brass, some of the copper conductor, ignited the case, and initiated the fire. Arson was ruled out.

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### Electrical, Arson, Fire



### D31 When Law Enforcement Relies on Some On-Board Vehicle Crash Data to Reconstruct a Crash Reconstruction in Criminal Prosecution, but the Overlooked Data is Exculpatory

*Billy S. Cox, Jr. \*, Billy Cox Group, Navasota, TX 77868*

**Learning Overview:** The goal of this presentation is to discuss the pitfalls of cherry-picking data obtained from downloading the crash data from a vehicle Event Data Recorder (EDR) using the Bosch® Crash Data Retrieval (CDR) tool in criminal prosecutions following a motor vehicle collision.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the use of EDR data from a vehicle that was struck from behind. The pre-crash data revealed critical pre-crash steering data that would indicate the intoxicated driver was not the at-fault driver in the collision.

The Bosch® CDR tool has been utilized for nearly 20 years by crash reconstructionists to assist in crash investigations. As vehicle technology has improved, the data that may be obtained following a crash has also become more detailed and beneficial, especially the pre-crash data. This presentation discusses the implications of ignoring some pre-crash data that is recovered in the imaging of the EDR.

In the subject collision, a RAM® 1500 pickup was being operated in the right, through traffic lane of U.S. 287 in Wilbarger County, TX, at 3:00 a.m. by a 20-year-old male. A Nissan® Pathfinder®, being operated in the through traffic lane was struck from behind, causing it to leave the roadway and roll over. The Nissan® Pathfinder® was occupied by two adults and three children, all of whom were unrestrained. One of the rear-seat occupant children sustained fatal injuries in the collision.

The driver of the RAM® 1500 pickup remained on the scene and attempted to assist the occupants of the second vehicle. He was interviewed by law enforcement at the scene and, after about one hour, voluntarily submitted to a blood alcohol test. His blood alcohol measured 0.11, which was over the Texas legal limit of blood alcohol concentration of 0.08 or more. He was arrested and subsequently tried for Intoxication Manslaughter. In Texas, a conviction for Intoxication Manslaughter has two prongs: (1) a person must be intoxicated, and (2) by reason of that intoxication, causes the death of another by accident or mistake.

During the initial investigation, the Texas Department of Public Safety officers downloaded the crash data from the EDR of the Nissan® Pathfinder® that was struck from behind. The EDR file contained data from two collision events; a front-to-rear collision and a rollover event, plus 5.0 seconds of pre-crash data for both events.

Based on the data contained in the EDR file, the reconstruction revealed that the impact speed of the RAM® 1500 pickup was 75mph, which was the speed limit on the highway. The speed of the Nissan® Pathfinder® was 39mph, yielding a closing speed of about 36mph.

The 5.0-second pre-crash data from the Nissan® Pathfinder® contained information for vehicle speed, service brake (On, Off), and vehicle steering input at half-second intervals, from -5.0 seconds to 0.0 seconds. Although the collision occurred in the center of the traffic lane that was occupied by the RAM® 1500 pickup, the pre-crash data indicated that the driver of the Nissan® Pathfinder® applied 2.0 degrees of left steer for 2.5 seconds, increasing to 10 degrees about 0.5 seconds prior to the crash and finally, 54 degrees of left steer at the time of the crash.

The pre-crash steering was ignored by investigating officers. A set of steering tests was performed with an exemplar vehicle prior to the trial and the steering input indicated that the driver of the Nissan® Pathfinder® executed a rapid lane change from the acceleration lane to the through traffic lane prior to being struck from behind. The reconstruction revealed that the rapid lane change by the driver of the Nissan® Pathfinder® was the cause of the crash and the intoxication of the driver of the RAM® 1500 pickup was not a factor in the crash.

Surveillance video from a convenience store nearby showed that the driver of the RAM® 1500 braked suddenly prior to striking Nissan® Pathfinder® in what could be considered a normal, unimpaired perception/reaction time of about 1.0 second in response to the rapid lane change. A jury of his peers found the driver of the RAM® 1500 guilty of driving while intoxicated, but found that he did not cause the crash.

This presentation will be highlighted with surveillance video, video from the steering tests, and reconstruction video from a subsequent fatality crash wherein the steering data was ignored by state police investigators in a two-fatality collision.

#### **Crash, Intoxication, Reconstruction**



### D32 Spoliation: Willful Loss of Evidence Crime in an Electrocution Case

*Helmut G. Brosz, BASc\*, Forensic Science International Group, Markham, ON L3P 3P2, CANADA*

**Learning Overview:** After attending this presentation, attendees will recognize that forensic electrical engineers and accident or crime scene investigators can play an important role in analyzing an electrocution scene as well as assisting first responders and criminalists in determining if willful spoliation could have occurred. The seizure of all relevant materials and artifacts is important and should not be left behind for someone else to destroy. Such artifacts can yield valuable evidence from an electrocution scene.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to an improved performance of criminalists and engineers involved in an investigation of an electrocution in a civil case. Can the willful loss of evidence lead to criminal indictments?

This case study covers an electrocution in a motor repair shop. The deceased technician was in the process of attaching two test clips to the 480-volt motor that had just been repaired. Upon making the two connections, he was electrocuted and fell to the floor. The assistant eyewitness working next to the deceased technician was of a religious/moral persuasion, which prevented him from applying any form of first aid including mouth-to-mouth resuscitation to the victim. The hard evidence from the scene consisting of test cables, with test clips and insulating boots attached, was turned over to the plaintiff's attorney for the deceased instead of being kept by the Authority Having Jurisdiction (AHJ) over workplace accidents.<sup>1</sup> The subject motor was not examined or retained.

A civil suit was filed by the plaintiff's lawyer against the manufacturer of the red insulating boots which covered the bare 50-ampere test clips. The complaint stated that the red insulating boots were defective and had some form of cuts, holes, or other unspecified defect that allowed contact between the hand and the bare 50-amp clip.

The plaintiff's attorney "lost" the boots, clips, and the wires to which they were attached. The only useful evidence were two photos take upon autopsy of the deceased's hand.

A reconstruction of the electrocution using porcine tissue and identical 50-amp clips demonstrated that the electrocution did not occur due to a slit, cut, or hole in the insulating boot as demonstrated by the burn patterns on the porcine tissue tests. Instead, it was clearly evident that the burn pattern on the deceased's hand and fingers was due to direct contact with the energized bare clips with the insulating boots having been shoved back so as to make it easier to apply the clips to the motor terminals. The deceased obviously forgot to turn off the power to the test clips before making his connections. The accident thus was the result of the deceased's own negligence, not a product liability case.

The law on spoliation (depending on the jurisdiction) says that mere negligence is not sufficient for a spoliation finding. What is required is: (1) that the evidence was destroyed; (2) that the evidence is relevant; (3) legal proceedings were pending; and (4) that the destruction of the evidence was an intentional act of the party, indicative of fraud or intent to suppress the truth.

In the opinion of the defense, spoliation occurred. No motion or hearing on spoliation took place. No criminal proceedings were initiated. The matter settled during trial.

**Reference(s):**

<sup>1</sup>. 2001 NFPA 921 sec.9.3.6.

**Spoliation, Electrocution, Evidence**



### D33 Criminal Engineering and Science Without Consequences: Why?

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**Learning Overview:** The goal of this presentation is to share with the forensic science community the concerns and questions regarding the prevalence of criminal engineering and science within the automotive and other product manufacturing communities. The often-nebulous distinction between negligence and criminal behavior by scientists, engineers, and their managers will be explored.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing important and enlightening information and discussion regarding examples of the lack of consequences for those making decisions that predictably, negatively impact public safety.

Why are engineers, scientists, and their managers within this industry not held criminally responsible for their defective designs? Routinely, we find instances where engineers, scientists, and their managers make decisions which negatively affect public safety. The unethical (criminal?) environment that enables “crooked” engineers and their managers to get away with exposing the public to defective designs for sundry reasons are a malignant part of American industry. Personal bonuses and increased corporate profit are basic factors motivating such unethical behaviors. Those responsible typically never suffer the consequences of their designs or the decision to deploy them. For example, the Volkswagen® executives currently under arrest/indictment for spoofing smog controls are for relatively insignificant offenses compared to the design and manufacture of predictably weak seats, slackening seat belts, fuel tanks that are in the crush zone at the rear or side of vehicles, inertially releasing seat belt buckles, exploding airbags, and perpetually weak roof and door latch structures, all of which have killed thousands of children and adults. An example of particularly grievous behavior by the automotive industry, in collusion with the United States Department Of Transportation (DOT), was a regulation governing roof strength (FMVSS 216), which was supposed to be a “temporary” standard, but remained in force for more than 30 years. In 1996, the DOT declared that FMVSS 207 was “flawed and inadequate,” yet automakers continue to design to this standard. Additionally, automakers continue to design seats to the flawed and inadequate FMVSS 207. The latest known instance of negligence that appears to rise to the level of criminal action, is with the Boeing® 737 MAX. Commercial aircraft defects are particularly notable in that hundreds of people die at once, instead of one or two at a time in ground vehicle crashes. If an automobile mechanic cut a hidden portion of a seat belt that later resulted in the failure of the belt and consequent injury/death, he would likely be held criminally liable for “sabotage” of the vehicle’s safety system. This is NOT the case for those that knowingly introduce defective products into the stream of commerce. Why should those who introduce dangerous designs at the engineering department or the board room, be treated any differently than an individual that intentionally disables a safety system?

In 1966, Dr. John Stapp, pioneer of American automotive safety testing and regulation, proposed that the automotive industry and its personnel be held individually criminally responsible for their actions that resulted in dangerous vehicle designs. Obviously, his insightful recommendation, based on well-known and documented human behavior, was rejected. It is this study’s position that the undue political and financial influence of industry and the lack of appropriate laws and/or enforcement are the reasons that such murderous behavior has been and continues to be allowed. Adults and children continue to needlessly die. Why?

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### Criminal Engineering and Science, Defective Engineering Decisions, Consequences-Negligent Engineering



### D34 Head and Neck Trauma: BioMedical Engineering Analysis of Entrapment Testing

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**Learning Overview:** The goal of this presentation is to inform the forensic engineering community about the importance of integrating various components of a system and testing the design as a whole to prevent real-world head and neck risks of entrapment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a better understanding of the factors that increase risk of injury/death due to entrapment.

**Introduction:** Nearly one million Americans live in some type of senior living community and that number is expected to double by the year 2030. Between 2003 and 2012, there were 155 bed rail-related deaths in which 129 of the 155 deaths occurred in people who were 60 years or older. With the increasing number of seniors residing in assisted living care, there is a greater need to focus on medical research to reduce health risks and provide preventative measures. This includes research of the mechanics of injuries that lead to fatalities. Entrapment of the head and neck can occur as a result of the body being wedged between two objects, creating a mechanism for asphyxiation. Biomedical engineering analysis is important to determine what can be done from a preventive perspective to ensure a safer environment for every patient in this increasing population.

**Case Study:** A facility failed to ensure that half-side rails were safely applied for an immobile resident who was using an alternating pressure mattress. This failure created a real and present danger when her head and neck became wedged between the half-side rail and mattress, resulting in fatality due to asphyxiation.

**Content/Methods:** Tests were performed using the Food and Drug Administration (FDA) -approved Bed System Measurement Device in the seven different possible entrapment zones that have been outlined by the FDA. Testing was conducted to determine if an alternating pressure mattress with the approved half-length bed rail passed or failed in the various zones/settings.

**Results:** During the analysis of entrapment incidents, this study revealed that the interactions between the separately designed components of the bed system were more concerning than any one component alone. Currently, the bed system is under-optimized and leads to entrapment possibilities. Error rates were analyzed and contributing factors of these error rates were determined.

**Conclusion:** The results confirm that there are risks for entrapment even in a bed system unit in which all components individually passed the FDA regulations for entrapment. This is due to their specific interactions between various bed components that were not properly designed and tested as a system. One contributing factor to this current issue is having different manufactures design the mattresses, bed frames, and bed rails that are frequently used interchangeably. The customized testing, in which all components of the system were tested as a unit, resulted in identifying entrapment risks and presented an unnecessary risk for users.

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#### Bed Rail, Entrapment, Mattress

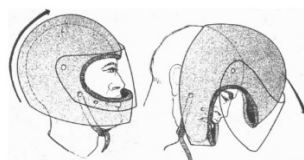
## D35 An Experimental Study of Inertial Mechanisms of Motorcycle Helmet Accident Retention Failures

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**Learning Overview:** This goal of this presentation is to demonstrate an experimental method for forensic study of motorcycle and motocross helmet inertial-induced detachment mechanisms during motorcycle crashes by using the National Highway Traffic Safety Administration (NHTSA) Pendulum anthropomorphic Hybrid-III head-neck test device for comparison of potential defective helmet retention systems.

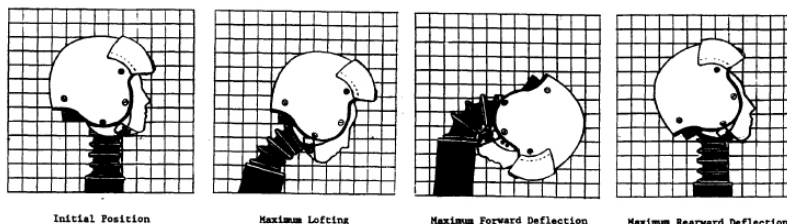
**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing an experimental method to evaluate the tendency of motorcycle and motocross helmets to be displaced from the head during inertial loading in a collision prior to the rider impact-contact with the ground.

Discussed in a prior American Academy of Forensic Sciences (AAFS) presentation, the protective capability of a motorcycle and motocross helmet is diminished or becomes non-existent if the helmet becomes displaced or ejected during an accident or sudden impact.<sup>1</sup> Earlier helmet research on displacement-detachment during crashes was published by Richards and Hurt, et al.<sup>2,3</sup> The United Kingdom Department for Transport reported “helmet detachment...in 10% to 14% of casualties.”<sup>4</sup> Restrained helmets can be significantly displaced or ejected off the head in motorcycle impacts, due to loose fastening of the chin strap and possibly a loose-fitting helmet. Hurt noted, “helmets properly fitted, retention system securely fastened, but the helmet is ejected.”<sup>3</sup> Richards diagrammed how properly fitted and securely fastened helmets can eject in frontal impacts where “the torso is slowed...but forward inertia of the helmet, the geometric attachment of the chin strap system, plus head rotation, allows the helmet to roll off the head before impact with another vehicle, and/or other fixed objects.”<sup>2</sup>



Manner in which helmet is detached at high speed, showing (left) normal position and (right) helmet pivoting forward on strap during accident.

In this current study, the NHTSA Pendulum anthropomorphic Hybrid-III head-neck test device was used for experimental evaluation of helmet detachment due to inertial unloading caused by the helmet design and retention system. Experimental methodology is based on earlier research by the United States Army Aeromedical Research Laboratory related to helicopter and military helmet retention during inertial impact loading.<sup>5</sup> The figure below, from reference 5, shows phases of head/helmet flexion (head bend toward chin), head/helmet extension (head/helmet bend reward), and helmet lofting from the head due to centrifugal forces.



The figure below shows video clips of similar testing recently conducted at IDIADA-KARCO on motorcycle helmets, using the NHTSA pendulum. New helmet models were impacted at different impact speeds (6.8 and 5.3m/s) and helmet sizes (M & L).



The figure above shows four inverted video clips (i.e., top of head in pendulum test is actually oriented toward floor) from different time frames of NHTSA pendulum impact testing at 5.3 meters per second (m/s) on a new, large-size motocross helmet mounted to a Hybrid-III 50<sup>th</sup>tile male head/neck. The upper left video clip is taken at the start of pendulum contact-impact just before the pendulum arm crushes an aluminum honeycomb pad. Prior to testing, the helmet



was tightly cinched to headform. The upper right video clip is 41msec after impact. The upper two video clips show initially, during the neck-flexion phase, the helmet becomes loose from the headform, lags behind the head rotation so the headform chin protrudes noticeably below the chin-bar, and the forehead rotates into the face opening of helmet. Detachment of the helmet from the headform is caused by the centrifugal force induced into the combined headform and flexible neck, which are affixed to the base of the rigid pendulum bar stopped suddenly by crushing of the deformable honeycomb pad. The next time clip at 176ms shows the helmet rotated back to almost the same position as the contact impact. Then the head-neck system goes into neck “extension” rotational mode such that the helmet is displaced from the headform and the chin again protrudes beneath the chin bar of helmet (i.e., 260ms clip). Although tightly cinched before impact, the helmet easily slides “loosely” back and forth over several degrees relative to the start of inertial impact.

In summary, the experimental methodology utilized by authors discussed in this presentation enables a more realistic, proper forensic evaluation of helmet system retention performance, regardless of compliance with limited government or independent safety standards, such as the “roll-off” test. It has been pointed out that existing helmet Standards FMVSS 218, British Standard 6658, and ECE Standard 22.05 do not adequately evaluate helmet retention deficiencies or potential head injury risks like the inertial testing employed in this study.<sup>5</sup> Typically, these standards only utilize a simplified “roll-off” test where a small weight is attached by a loose cable to the rear edge of the helmet mounted on an inclined metal headform. Weight is then dropped about 0.6 meters to cause limited “sudden jerking motion” to helmet. This method does not address the more realistic “flexion” and “extension” kinematics achieved with the pendulum test method.

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### Motorcycle Helmet, Retention, Impact

### D36 The 1921 Death of Nino Martoglio, Pirandello's Mentor: A Cold Case and Literature Review

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**Learning Overview:** The objective of this presentation is to establish the cause of death of a man who lived 100 years ago through a multidisciplinary approach of forensic pathologists, anthropologists, and software engineers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the importance of an analytical and scientific method that can change the cause of death even in a case from 100 years ago. Anthropological investigations, forensic radiology, and engineering informatic study could reconstruct the bone lesions and the possible kinematics of the fall.

Forensic pathologists who deal with blunt force injuries to the head are often asked to determine whether the trauma is related to a fall or induced by blows. This is a challenge for forensic pathologists that have to discriminate falls and blows in blunt head trauma. A recent and 1800s literature review was performed. Guyomarc'h, Pierre et al. demonstrated that in the discrimination of falls versus blows, the Hat Brim Line (HBL) rule is one of the most useful single criteria.<sup>1</sup> According to this rule, an injury located at the level where the brim of a hat would lie is more likely the result of a fall, while a blow would generally produce a wound above this line. According to Petaros et al., falls from lower heights (<3.5m) are characterized by occipital, temporal, and parietal fractures and produce lesions like scalp lacerations and linear or radial fractures of the skull.<sup>2</sup> In the height group (4–10m and 10.5–30m), there is a more generalized distribution with fractures localized in the upper/middle body, lower body, and throughout the whole body. Unlike falls, blunt trauma often causes injury above the HBL and depressed fractures (type IV according to Guyomarc'h, Pierre et al.) that reproduce the shape of the used object.<sup>1</sup> Lacerations at the injured site and defensive lesions at the level of the hands are present as well. A review of 3D models for simulating head impact biomechanics was performed, too. O'Riordain, K. et al. thought MADYMO software (Mathematical Dynamic Models-TNO, 1999) established that fall from the lower height of <3m caused a right frontal linear skull fracture, right frontal extradural hematoma, left posterior temporal basal contusion.<sup>3</sup>

**Case Report:** The case reported happened nearly 100 years ago. Antonino Martoglio is a Sicilian historical figure, a famous poet, writer, one of the first film directors, and Pirandello's mentor who died in mysterious circumstances. The morning of September 16, 1921, the cadaver of the well-known Sicilian poet was found in the lift shaft of the hospital. The night before, he left the room where his son was hospitalized, and went to an isolated under-construction area of the same building. The lift shaft was 3.35 meters high and measured 1.80x1.30 meters. With the approval of the prosecutor's officer, an engineer inspected the building and an external examination was conducted by the local medical examiner. The medical examiner reported an extended medial-frontal bruise with an irregular lacerated-contusion wound and a depressed fracture of the frontal bone. The medical examiner established that the death was due to a violent frontal trauma resulting from a fall from a low height (<3.5m); for this reason, he established it was not necessary to proceed with an autopsy. An 1800s literature review about falls from different heights and related lesions support the hypothesis that those injuries reported can exclude a 3.35m precipitation of a corpse and an autopsy.<sup>4</sup> Moreover, depressed fractures were described exclusively in blunt traumas. The data that emerged from the review were sent to computer scientists who performed an engineering informatic study. Through computer software, informatic engineers evaluated all the possible injuries involved by a fall of 3.35m and established that the most affected region was the head. The 3D computer simulation will be presented.

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4. *Lezioni di Medicina Legale - Francesco Puccinotti - Seconda Edizione Napoletana - 1858.*

#### Fall From a Height-Blunt Trauma, Engineering Informatic Study, Literature Review





### D37 Skull Fracture Risk: An Experimental Comparison Between Elastic (Drone) and Inelastic (Wood Block) Impacts Focused on Fracture Type and Tolerance Using Instrumented Postmortem Human Subjects (PMHS)

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**Learning Overview:** After attending this presentation, attendees will have an appreciation for interdisciplinary research in skeletal trauma analysis, specifically focused on the cranial vault. Attendees will learn how to conduct experimentally driven biomechanical validation of skeletal trauma for forensic engineering purposes and will understand the importance of utilizing experimental data to better understand the differences in fracture type and threshold between elastic and rigid blunt force impacts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the need for interdisciplinary biomechanical analyses of skeletal trauma in the cranial vault due to both elastic and rigid blunt impacts. Increased understanding of the differences in fracture tolerance and type due to elastic and rigid blunt force trauma will serve to improve skeletal trauma analyses in forensic engineering.

Currently, there are over one million recreational drones in the United States, with forecasts predicting increases to more than three million by 2021.<sup>1</sup> Originally, the Federal Aviation Administration (FAA) regulations limited drones to weights below 55lbs and top speeds below 100mph.<sup>2</sup> Recently, the FAA has begun allowing more widespread Unmanned Aircraft Systems (UAS) operations through a waiver program. As more drones populate the airspace and more lenient regulations are enacted, there is a need to understand and quantify the risk associated with drone impacts. To date, there have been several documented cases of concussion and whiplash injuries resulting from UAS impacts as well as one case of an Abbreviated Injury Scale (AIS) 3 level skull fracture.<sup>3</sup>

The focus of this study was to impact Postmortem Human Subjects (PMHS) over a range of drone head impact scenarios (including a wooden block) to: (1) investigate differences in fracture type between drone (elastic) and wooden block (inelastic) blunt impacts; and (2) assess current automotive-based injury criteria to predict the fracture thresholds of the cranial vault. The experimental variables included: drone impact orientation, drone vehicle type, and impact velocity.

The tests were conducted by using a catapult device to accelerate the drones and wooden block into five separate PMHS, with each PMHS receiving multiple impacts focused on separate bones of the cranial vault. A total of 41 blunt impacts were conducted using five different drones and a wooden block at speeds up to 22m/s. The PMHS were instrumented with strain gauges on the bones that comprise the cranial vault and two different arrays of accelerometers and Angular Rate Sensors (ARS) mounted to the skull to measure six-degree of freedom head kinematics. In-between each impact, X-rays of the head were taken to document if a fracture had occurred due to the preceding drone or block impact. Following the test series for each PMHS, an anatomical dissection was completed to document all fractures that occurred to the cranial vault due to the blunt impacts.

Of the 35 elastic drone impacts carried out, one AIS 2+ injury was observed: a 13cm linear skull fracture in the frontal bone resulting from a Phantom 3 impact. In addition, three rigid wood block tests all resulted in fractures to the cranial vault, one of which was an AIS 4 severity injury. Kinematics measured during PMHS tests indicated that automotive injury metrics may not be able to accurately predict skull fracture due to drone or elastic blunt impacts. To assess the risk of skull fracture, the Head Injury Criteria (HIC<sub>15</sub>) and its associated risk curve were compared to PMHS head kinematics.<sup>4</sup> Based on the HIC<sub>15</sub> criteria, five drone impacts had greater than an 85% probability of causing a skull fracture; yet only one skull fracture was observed. Additional investigation is needed to determine appropriate criteria or limits to be used for predicting the severity of head fractures due to elastic blunt impacts.

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#### Skull Fracture, Injury Biomechanics, Blunt Force Trauma



## D38 Cervical Spine Injury of Postmortem Human Subjects in Rear-End Impacts

*Yun-Seok Kang, PhD\**, The Ohio State University, Columbus, OH 43210; *John H. Bolte, PhD*, The Ohio State University, Columbus, OH 43210

**Learning Overview:** After attending this presentation, attendees will have an appreciation for interdisciplinary research in skeletal trauma analysis, specifically regarding the cervical spine. Attendees will learn why cervical spine injury still occurs in rear-end impacts based on experimentally driven biomechanical responses for forensic engineering purposes and understand the importance of utilizing biomechanical data to investigate potential injury mechanisms of the cervical spine in rear impacts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the need for interdisciplinary biomechanical analyses of trauma in the cervical spine in moderate-speed rear-end impacts. Biomechanical data for intervertebral kinematics and injuries of the cervical spine in moderate-speed rear-end impacts will help attendees understand a potential injury mechanism for the cervical spine and ultimately improve injury analyses in forensic engineering.

In the United States, claims of cervical spine injuries (e.g., whiplash) in rear impact collisions are very common and result in enormous societal cost, with estimates of \$2.7 billion annually.<sup>1</sup> Even though cervical spine injuries can result from frontal and rear impact crashes, their risk in rear impact crashes is twice that of frontal impact crashes.<sup>2</sup> Several types (e.g., muscles, ligaments, facet joints, intervertebral discs, nerves) of cervical spine injuries in rear-end impacts have been reported.<sup>3</sup> Although extensive research using both volunteers and Postmortem Human Subjects (PMHS) has been conducted with a focus on cervical spine injuries due to low-to-moderate rear impacts, relevant anatomical sites, clinical evidence for injury, and the mechanisms of injury are still unclear. Therefore, the aim of this study was to investigate intervertebral kinematics and injuries to the cervical spine of whole body PMHS by exposing them to moderate-speed rear-end impacts in both experimental and production seats.

Twenty-one rear impact sled tests using 15 PMHS (73 ± 12 year old, 178.6 ± 6.3cm of stature, and 78.7 ± 6.2kg of weight) were conducted with Delta Vs ranging from 17 to 24km/h (Federal Motor Vehicle Safety Standard [FMVSS] 202a, Japan New Car Assessment Program [JNCAP], and 10.5g/24km/h). PMHS were placed in both experimental and production seats that exhibited seat back rotations ranging from 5 to 35 degrees. Head kinematics of the PMHS were measured using six accelerometers and three Angular Rate Sensors (ARS) mounted on the head. In order to measure both gross and intervertebral kinematics of the cervical spine, three accelerometers and three ARS were installed on the anterolateral aspect of the cervical vertebral bodies using custom wing mounts. A three-point belt without a pretensioner or a load limiter was used to restrain the PMHS on the seats, with initial belt tensions of 17.8N (4lb) for the lap-belt and 26.7N (6lb) for the shoulder-belt. The initial seat-back angle was approximately 25 degrees from vertical.

Results show that the intervertebral rotations of the cervical vertebrae represented flexion (i.e., relative forward rotation of upper vertebra relative to lower vertebra), although all cervical vertebrae rotated rearward in the global coordinate system. This relative forward rotation occurred in all three moderate speed conditions, at all intervertebral levels, for both experimental and production seats: 6.5 ± 3.6 and 4.2 ± 2.8 degrees for C2/C3, 6.2 ± 4.2 and 4.0 ± 2.3 degrees for C3/C4, 10.2 ± 5.8 and 6.3 ± 3.8 degrees for C4/C5, 7.6 ± 4.7, 6.1 ± 3.5 degrees for C5/C6, and 6.7 ± 3.8 and 5.8 ± 3.2 degrees for C6/C7, respectively. Several laxities between cervical vertebrae were discovered and identified from post-test dissection. The cervical flexion kinematics observed in this study using both experimental and production seats are not representative of traditional neck extension injuries, but results from this study indicated that intervertebral flexion kinematics may be regarded as an additional potential injury mechanism of the cervical spine during moderate-speed rear-end impacts.

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### Cervical Spine, Injury Biomechanics, Rear-End Impacts

## D39 Head Injury Criteria (HIC) Scaling for Assessing Closed Head Injury Risk

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**Learning Overview:** The goal of this presentation is for attendees to learn how to scale HIC.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a method for estimating head injury from available crash test.

Due to the expense associated with automobile crash testing, it is desirable to develop an appropriate scaling technique in order to utilize existing test results to predict the head/brain injury risk at a particular crash severity.

All head impacts involve linear and angular accelerations, as the two head accelerations are inextricably coupled by impact vector and head-neck structures.<sup>1,2</sup> Concussion risk has been correlated with both peak linear and angular head acceleration.<sup>3</sup> Unfortunately, angular parameters are not measured in standardized automotive crash or sled testing.

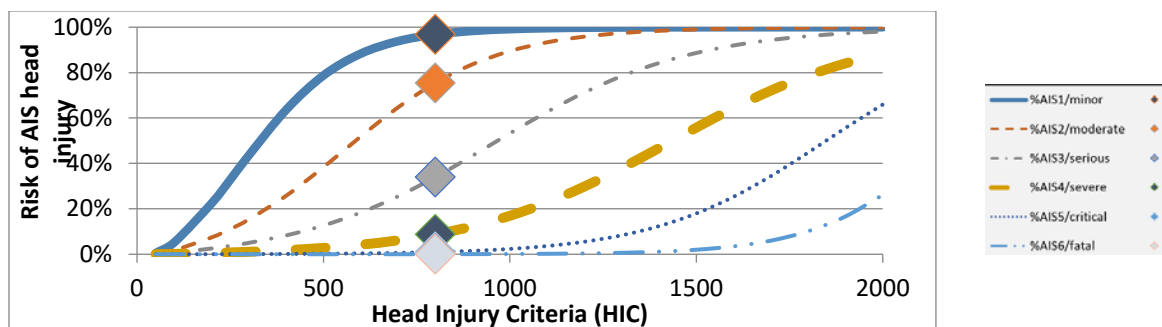
The Head Injury Criterion (HIC) is based upon the linear resultant head center of gravity acceleration. The HIC is the maximum calculated value over a time frame where  $t_2 - t_1 = 15$  or 36 msec using the formula shown in Figure 1.

$$HIC = \left\{ \left[ \frac{1}{t_2 - t_1} \int_{t_1}^{t_2} a(t) dt \right]^2 (t_2 - t_1) \right\}_{max}$$

**Figure 1. Head Injury Criterion<sup>4</sup>**

Excluding combined linear-angular and tissue strain based methods, HIC has been found to be the best predictor of mild traumatic brain injury when compared to other methods, such as the Gadd Severity Index, linear acceleration, and rotational acceleration.<sup>5</sup> HIC is the current worldwide standard for assessing head impact severity and head/brain injury risk and is inherent in Federal Motor Vehicle Safety Standards.

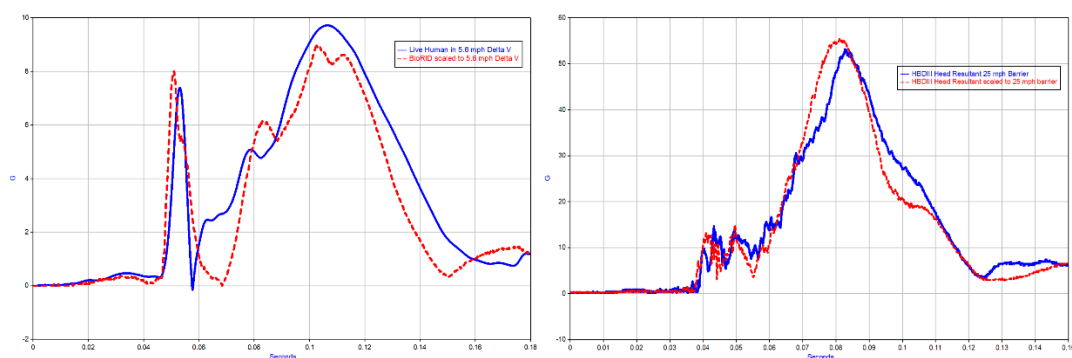
The Abbreviated Injury Scale (AIS) is a standardized system to describe injury severity that ranges from AIS 1 to AIS 6, representing minor through fatal/untreatable injury.<sup>6</sup> Prasad and Mertz set forth a risk curve for severe, AIS  $\geq 4$ , head or brain injury as a function of HIC.<sup>1</sup> The National Highway Traffic Safety Administration (NHTSA) later expanded the risk curves for all AIS levels.<sup>7</sup> As shown in Figure 2, an event with an HIC of 1,000 is considered to have about a 1 in 6 chance of a severe head or brain injury. Below 50, there is effectively no chance of even the most minor (AIS 1 or 2) head injury.<sup>8</sup> Volunteer studies are routinely conducted in a range that produce HIC's below 50 without consequence.



**Figure 2. Probability of Head Injury Severity as a function of HIC<sup>8</sup>**

Delta V is a common measure of crash severity. Proportional scaling the HIC for Delta V is not appropriate since the HIC is non-linear for head acceleration. However, if head acceleration varies linearly with Delta V, then Delta V can be used to scale head accelerations from a crash test to that of an occupant of an accident vehicle. The head injury risk for that occupant can be estimated using the HIC calculated from the scaled head accelerations, then applied to the Prasad-Mertz curves to estimate head/brain injury risk.

Crash tests at two different Delta Vs, but with comparable vehicles, occupants, restraints, seat positions, etc., are uncommon. An example from a 10mph rear-end sled test scaled down to live human exposure to a rear-end car-to-car impact with same year, make, and model of vehicle, seat belt use, and active head restraint deployment is shown in Figure 3 (left). The live human that was close in stature to that of the 50<sup>th</sup>-percentile Rear Impact Dummy (RID). Another example is scaling a 25mph barrier impact to a 30mph barrier impact with the same year, make, and model of vehicle, both with an unrestrained 5<sup>th</sup>-percentile female HBDIII dummy with the passenger seat adjusted to the same position, shown in Figure 3 (right).



**Figure 3. Results of scaling a rear sled test to a bumper-to-bumper impact (left) and frontal barrier crash tests (right)**

By scaling the resultant head acceleration, the estimated HIC<sub>15</sub>'s compared favorably but somewhat over-estimated the actual values from the lower speed crash tests. As would be desirable in practice, in these examples as many parameters as possible were kept constant. Inherent in this technique is the assumption that the collision durations are approximately the same so that the vehicle and head acceleration varies approximately linearly with Delta V.

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#### HIC, Scaling, Risk



## D40 Forensic Motor Vehicle Accident Investigation

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**Learning Overview:** After attending this presentation, attendees will have gained an awareness of current knowledge regarding the relationship between automobile body paint color and motor vehicle accident prevalence and severity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the importance of automobile body paint color and its relationship to motor vehicle accident prevalence and severity.

Engineers have long sought to improve occupant safety by inventing vehicle crash avoidance or crash energy mitigation strategies, structures, systems, or devices. Numerous studies have shown that high center-mounted third brake lights, stability control systems, seat belts, and crush zones (to name a few) help prevent accidents or reduce injury severity. However, few studies have addressed automobile body paint color as a factor. This is unfortunate because automobile body paint color is among the simplest and least expensive means of enhancing vehicle visibility. Despite public demand for increased vehicle safety, automobile body paint color and its relationship to motor vehicle accident prevalence and severity is largely unaddressed.

Color of consumer goods has deep roots in society. From the days when only quality fabrics could take dark dyes to Henry Ford's statement that "...the customer could have any color wanted as long as it was black," color has played a role in consumer choice. Ford's "black" strategy worked for low-cost mass production. Black (and gray) also work as important accent colors in the clothing and automobile industries. Consumers feel these colors suggest dignity, formality, solemnity, and power.

Mid-century consumers witnessed a wider array of automobile body paint colors to show product distinctiveness (two-tone color schemes), owner personality (bright psychedelic reds, pinks, yellows), or increasing technological sophistication (metallic or pearlescent colors enabled by metal particle suspension or multi-layering). Recent (2018) data shows consumer preferences for new automobile paint colors: white: (19.3%), silver (18%), black (12.4%), dark blue (11.4%), dark gray (7.5%), red (7.1%), dark green (6.7%), and brown (5.1%). Since 2006, white has been the primary color choice of the American automobile consumer. The popularity of this color is also increasing internationally.

So what's color got to do with forensic analyses of automobile crashes? Plenty, according to a decade-old study by the Monash University Accident Research Centre. They assessed the relationship between vehicle color and crash risk. Actual crash data reported to police in two Australian states were stratified by vehicle type, light conditions, and legal jurisdiction. The results showed a statistically significant relationship between vehicle color and crash risk. White vehicles had the least reported crashes, and thus the risk of all crashes were indexed to such vehicles. Compared to white cars, black, blue, gray, green, red, and silver were associated with greater crash risk. Crash risk associated with other colors were statistically indistinguishable from white, but this may be a Type II statistical error. The association between vehicle color and crash risk was strongest during daylight hours when relative crash risks were as much as 10% greater for black vehicles compared to white vehicles. The data also suggested that environmental factors are potential covariates modifying the relationship between vehicle color and crash risk. Crash severity was also related to vehicle color: lower visibility vehicle colors were associated with greater risk of more severe crashes.

Strength of the relationship between various automobile body paint colors and crash prevalence or severity was unclear. Although the Insurance Institute for Highway Safety and federal government studies estimated that crash reduction due to Daytime Running Lights (DRLs) is 3% to 5%, DRL data is distinct from automobile paint color data because other factors, such as vehicle shape, size, orientation, surroundings, backlighting, etc., may also be involved in relationship with crash prevalence and severity.

The Uniform Police Traffic Collision Report form includes vehicle color; however, Red, Orange, Yellow, Green, Blue, Indigo, and Violet (ROY G. BIV) type entries are generally insufficient given the wide variety of paint colors (and visibility) with the same name. The investigating forensic engineer should also record the manufacturer's paint code number to identify vehicle color. This code is typically a three-digit number printed (embossed) on the metal specifications tag riveted to the driver's B-pillar. Colors of the surrounding environment at the time of the accident, given the perspective and lighting conditions seen by the observer, also must be documented and considered.

### Forensic Motor Vehicle Accidents, Motor Vehicle Body Color, Automobile Visibility



### D41 Body-Mounted Camera Motion Analysis—Accuracy and Validation of 3D Camera Match Solutions

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**Learning Overview:** After attending this presentation, attendees will have an understanding of dynamic camera analysis, applying photogrammetric techniques to validate results, and the various challenges faced by investigators, engineers, and scientists in solving for evidence in a moving camera system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing forensically sound methodology in the photogrammetric analysis of video evidence from body-mounted camera systems, using widely available tools from both the accident reconstruction and motion picture industries.

The analysis of video evidence captured by a moving camera system is one of the most difficult tasks faced by reconstruction professionals. While static photogrammetry techniques can be utilized with individual video frames, the vast number of image frames in a single video file make this method both time- and cost prohibitive. High frame rates (images per second), camera lens distortion, low-light scenarios, and image compression artifacts further complicate solutions using traditional photogrammetry. The motion picture industry heavily relies on the ability to solve for moving cameras in video compositing—that is, combining live video footage with rendered 3D objects. In movie production, the solution of moving camera footage is referred to as match-moving or camera-tracking. In this study, camera-tracking techniques are employed to solve for the motion of two of the most common sources of video evidence: Axon® body cameras (commonly worn by peace officers), and GoPro® action cameras (commonly worn by civilians and athletes). This report presents a study validating match-moving techniques used to solve for dynamic camera positions and objects within video frames.

In the first phase of a validation study, body-mounted action cameras are used in an environment optimized for photogrammetry: an indoor laboratory with uniform overhead lighting, a checkerboard floor texture, and a pre-defined motion capture area. The human test subject is equipped with a body-mounted camera and a biomechanical 3D motion capture suit that records body segment locations, speeds, rotations, and accelerations. The human motion is also recorded by static overhead video cameras. Key camera locations in the dynamic sequence are solved using static terrestrial photogrammetry. The statically solved camera positions are then compared with the solutions obtained using match-moving/3D camera tracking and the biomechanical motion capture suit.

After validating the optimized camera solutions using match-moving software, the study is extended into a non-optimized environment. The outdoor scene selected is a typical mixed-use industrial area, with large variations in sunlight/contrast, and very few idealized tracking references. The human test subject is instrumented with the motion-capture suit and a body-mounted camera and put through a series of motions, including walking, running, backing away from another human, and climbing a fence. The camera motion is solved using match-moving/3D camera tracking, and the solution is compared with the validated motion capture data set.

The results of this study show that 3D camera tracking or match-moving software can be used accurately to automate body-mounted camera motion solutions. Recommended guidelines include specific (key frame) static photogrammetry solutions to verify important locations in video footage. Pitfalls and other potential issues with frame rate, lens distortion, rolling shutter effects, and artifacts are discussed in detail. The presented methodology can be extended to both the criminal (officer-involved incidents) and civil (collision analysis) domains of reconstruction.

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#### Photogrammetry, Video, Analysis

## D42 3D Computer Photogrammetric Analysis of Multiple Surveillance Cameras Synchronized Into a Single Data Set to Track the Movement of a Vehicle

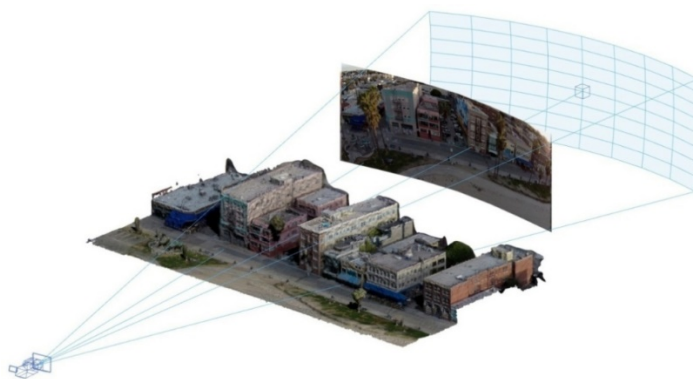
Jorge Mendoza, BS, ME\*, *Litigation Animation, Inc, San Jose, CA 95120*

**Learning Overview:** After attending this presentation, attendees will have learned the process of using a laser scanner, aerial mapping drone, 3D animation software, aerial mapping software, and computer reverse projection photogrammetry to reconstruct the vehicle’s velocity and trajectory, which will be discussed.<sup>1</sup> Attendees will understand how the final photogrammetric solution is composited into ground photos and aerial images used to generate accurate exhibits for trial.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sharing the procedures, equipment, and software used to combine multiple surveillance video sequences into a single 3D computer model for photogrammetric analysis, and from the results create a 3D animation file for trial.



Through the case study of *People v. Nathan Campbell* attendees will learn how photographic evidence, 2D physical evidence diagrams, 3D laser scan data, 3D optical scan data, aerial images, and the footage from multiple surveillance cameras were integrated into a single 3D model for photogrammetric analysis of a vehicle’s movement.<sup>1</sup>



On August 3, 2013, a vehicle entered the Venice Beach boardwalk at Dudley Avenue and traveled approximately 600 feet before exiting at Sunset Avenue. The driver, Nathan Campbell, claimed that he accidentally put the vehicle into drive entering the Ocean Front Walk. The vehicle struck 17 people, killing 1 person. Mr. Campbell claimed that the vehicle, a 2008 Dodge® Avenger®, had malfunctioned as he repeatedly attempted to stop the car. As the vehicle traveled down the boardwalk striking people and various objects, it generated an extensive debris field. The vehicle’s movement and collisions were captured by several shop surveillance cameras located on the boardwalk. Physical evidence such as skid marks, pieces of the vehicle, broken vendor equipment, bicycles, trash cans, blood splatter, and the decedent’s body location were valuable bits of information in refining the vehicle’s position at those locations.



After reviewing crime scene photos, surveillance video, laser scan data, police reports, and witness statements, a site visit was made. An additional optical scan was conducted by flying an autonomous drone with Pix4D® aerial mapping software. After conduction of a pre-visualization of the crime scene, key vantage points were identified and photographed from the ground and the air. These key images would later be composited into the final 3D

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photogrammetric model for the creation of trial exhibits. The surveillance cameras were located, photographed, and their positions on the buildings measured. The subject vehicle was then photographed, examined, and its dimensions documented for modeling.

Having completed the field work, a 3D wireframe model of the scene and a 3D surface model of the vehicle were developed. At this point, the vehicle surface model, point cloud, and surveillance videos were all imported into 3D Studio Max® for analysis. The virtual cameras were created for each video sequence and positioned in the 3D environment, then the camera was matched to their respective video. Next, the vehicle movement from each video sequence was tracked frame by frame and the 3D vehicle animated to each position. Finally, courtroom trial exhibits were created. Mr. Campbell was sentenced to 42 years to life in state prison.



The process of using a laser scanner, aerial mapping drone, 3D animation software, aerial mapping software, and computer reverse projection photogrammetry to reconstruct the vehicle's velocity and trajectory will be discussed.<sup>2</sup> The attendees will understand how the final photogrammetric solution is composited into ground photos and aerial images used to generate accurate exhibits for trial. Aerial images captured by drones are extremely valuable in that they are capable of capturing large areas that provide a clearer understanding of an event. Attendees will learn how to treat the scene as if it were a Hollywood movie set. Attendees will understand the value of performing a pre-visualization of the scene to capture key ground and air images from select vantage points that will later be camera matched with the final 3D photogrammetric model to create exhibits for trial.



Image resolution and quality are key elements that directly impact the accuracy of the analysis. The benefits of computer-based photogrammetry over traditional modified camera photogrammetry will be made evident.

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### Computer Photogrammetry, Aerial Mapping, Laser Scan



### D43 Vehicle System Forensics and Criminal Investigations Involving Automobiles

Wesley Vandiver, BA\*, Collision Forensics, Inc, Temecula, CA 92592

**Learning Overview:** After attending this presentation, attendees will have been exposed to the rapidly growing science of vehicle system forensics.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by disclosing how the discovery of vehicle system data and the development of tools for data acquisition have opened an entirely new source of key evidence in investigations involving automobiles.

Attendees of this presentation will be exposed to the rapidly growing science of vehicle system forensics. Like other forms of digital forensics, the acquisition of data from automobiles centers on the retrieval of digital data from various forms of storage media, and there is an overlap with some data from familiar digital forensics sources, including phone data (phone call histories, call logs, contacts, and text messages). However, unlike traditional digital forensics, the data acquired from vehicles can include key evidence regarding historical vehicle whereabouts (breadcrumb trails), vehicle velocities, hard acceleration/braking, traction events, doors opening or closing, gear shift selector changes, etc. Examples of the successful use of vehicle system data in criminal cases will be presented, including the case described below in which the data were key evidence in a vehicular murder case prosecuted in the United Kingdom.

The storage of digital data in automobiles is not a new concept, but the usefulness of such data in criminal investigations has developed more recently. Digital data in automobiles began with diagnostic data used for the purpose of maintenance and repair, along with the control and monitoring of emissions. Apart from diagnostic data, many vehicles have recorded crash data since the late 1990s and early 2000s. Event Data Recorders (EDRs) have advanced over time to record tri-axial acceleration traces and pre-crash vehicle data. The latest, and perhaps most powerful, entry into the available data from automobiles is the development of vehicle system forensics.

On August 5, 2017, there was an altercation at Bar Form, a bar in Enfield, United Kingdom. The altercation moved outside to the parking lot, where Bradley Clifford, 24, had parked his Ford® Mustang®. Mr. Clifford was arguing with Jashua Francis, 19, regarding his girlfriend. The two men agreed to go to a car park for a fist fight. Mr. Clifford got in his Mustang® and Mr. Francis got on his moped. Riding pillion on the moped was Sobhan Khan, 18, a friend of Mr. Francis. As the parties drove away, Mr. Khan smashed a bottle on the side of the Mustang®. Mr. Clifford then pursued the moped at high speed through the city streets of Enfield until finally catching it and ramming it from the rear. The impact ejected both Mr. Khan and Mr. Francis. Mr. Khan died from his sustained injuries. A crime scene image, used with the permission of the metropolitan police, is shown as Figure 1.

The Mustang® was manufactured with a Ford® Sync Generation 3 infotainment system, which yielded direct evidence of the crime, including vehicle track logs (breadcrumb trails) and velocity logs derived from Global Positioning System (GPS) data, as well as wheel speed data reported on the vehicle's Controller Area Network (CAN) bus. Equally valuable was the indirect evidence the system provided. The breadcrumb trail in the data gave investigators a highly accurate trace of the pursuit path. With this, police personnel searched the surroundings along this path for sources of video evidence (i.e., security cameras). With the prevalence of security cameras in today's world, investigators were able to identify numerous cameras that captured video footage of the chase. The gathered video clips provided corroborating evidence of vehicle speed, but also the aggressiveness of the pursuit, the vehicle spacing at points along the route, evasive actions by the moped, etc. The volume of evidence that included the direct vehicle system data and the indirect evidence that resulted from it overwhelmingly disputed any claims that the impact and resulting death of Mr. Khan were accidental.



Figure 1. Crime scene image, courtesy of the metropolitan police, United Kingdom

### Vehicle System Forensics, Infotainment and Telematics, Vehicle Investigations



### D44 The Development of an Intelligent Mobile Application to Enhance the Quality of Latent Fingerprint Acquisition

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**Learning Overview:** During this presentation, attendees will be introduced to an intelligent mobile application that is developed to assist the process of latent fingerprint acquisition. The application uses integrated cameras of smart phones to capture latent fingerprints and is able to indicate the quality of such fingerprints with both a graphical color-map and a numerical reliability score in real time. As such, it assists crime scene investigators to capture the optimal black-on-white fingerprint image.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility of mobile computing and the potential to integrate Artificial Intelligence (AI) into crime scene investigation and latent fingerprint acquisition. The mobile application runs real-time algorithms to identify usable and unusable areas of a latent fingerprint image and, furthermore, leverages techniques, such as Augmented Reality (AR), to provide graphical indicators (i.e., green and red color-maps) to inform the investigator of the quality of such images. This application makes it possible for investigators to determine the optimal camera angles, distance, illumination, etc. during the latent fingerprint acquisition and, therefore, enhance the quality of the acquired latent fingerprint image.

Latent fingerprint image capturing used in the forensic investigation field requires that quality images be taken in order to accurately match fingerprints to those stored in a database. The goal of this project was to implement a mobile application to aid forensic investigators in the latent fingerprint acquisition process. In a typical use scenario, the investigator points the camera of the smart phone to a latent fingerprint, and the application is able to recognize and analyze the latent print in real-time and display a graphical indicator (i.e., green/red color-map) for useable/unusable fingerprint areas, as well as a numerical score evaluated based on the overall fingerprint quality. The acquired latent fingerprint image can be directly send to a remote Automated Fingerprint Identification System (AFIS) for latent fingerprint searching or matching.

The application development process was sectioned into three major components: *mobile application* development, *reliability score algorithm* development, and *client-server system* development. The *mobile application* development creates a camera application and photo gallery, which allows latent fingerprints to be captured and stored on the smart phone. The *reliability score algorithm* development is currently based on and modified from the SourceAFIS, an open-source fingerprint analysis and matching project. The algorithm provides the basis for a green/red color-map overlay to the application that indicates the usable and unusable areas of a captured latent fingerprint image. The *client-server system* is also developed based on the SourceAFIS, which accepts images acquired from the mobile application, and is able to conduct 1:1 fingerprint matching, or 1:N fingerprint searching. It is also noteworthy that the modular design of this project is flexible enough to allow various algorithms to be easily exchanged, and one of the research goals is to develop and integrate AI-aided algorithms to enhance the quality of latent fingerprint development and acquisition during crime scene investigation.

All in all, this mobile application serves the purpose of being informative to forensic investigators during the process of latent fingerprint development, analysis, and acquisition.

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#### Latent Print, AFIS, Crime Scene Investigation

## D45 The Bisected Man: An Uncommon Pattern of Injury in a Fatal Motorcycle Crash

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**Learning Overview:** The goal of this presentation is to present an unusual case of a fatal motorcycle crash.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by evidencing the importance of the medicolegal and engineering evaluation in an uncommon motorcycle fatality crash.

Powered two-wheel riders are one of the most vulnerable groups of road users. The object most frequently struck in an accident is a passenger car, followed by the roadway itself, either as a single-vehicle accident or as an attempt to avoid a collision with another vehicle.<sup>1</sup>

The most common sites of injuries in motorcycle crashes are the head—even when a helmet is worn—and the limbs, but lesions of the thorax and the abdomen are described as well.<sup>1-3</sup> Typical lesions described in motorcycle accidents are basicranium fractures, like the so-called “motorcyclist’s fracture” or the “ring fracture” around the magnum foramen.<sup>4</sup> Other typical lesions are the “fuel tank injury,” produced by the impact of the inguinal region against the fuel tank.<sup>5</sup> The upper limbs frequently present hematoma of the palms and fracture of the wrists due to the impact against the handlebars, which are strongly grabbed during the crash.

**History:** This case report concerns the death of an 18-year-old man involved in a fatal motorcycle collision against a very large loaded truck, the size of which exceeded the transversal size of the lorry. The collision between the motorcycle rider and the loaded truck caused the transection of the rider’s body at the abdominal level.

The external examination revealed a severe lesion of the abdomen with partial extrusion of the intestine and the mesentery. This lesion was accurately measured to compare its characteristics with the truckload: it was 39cm long and 25cm away from a transversal plane passing through the hips. It extended from one hip to the other, and had a transverse major axis. On the upper part of the laceration, there was a net-shaped excoriation. The area also presented multiple linear excoriations. The upper limb presented millimetric excoriations and lacerations and the compound and exposed fracture of the left wrist. The lower limbs presented diffuse excoriations. After the removal of the helmet, which was correctly worn, no external lesions were documented on the head.

The autoptic examination revealed several thoracic fractures, involving all the ribs bilaterally. There was a gastric laceration in the abdomen, though no gastric material ran into the abdominal cavity. There was a huge laceration of the liver, spleen, and left kidney. The thoracic aorta was lacerated. There was the complete transverse fracture of the bodies of the first dorsal vertebra and the second lumbar vertebra. The brain section revealed the subarachnoid hemorrhage of the left temporal lobe, cerebellum, and pontomesencephalon. The cervical examination revealed the linear fracture of the body of the fifth vertebra.

A combined engineering and medicolegal evaluation was performed to investigate the exact dynamics of the road accident. The two vehicles were examined, highlighting the damages of the left anterior part of the motorcycle and some stains on the loaded truck, that were the same color as the motorcycle. The lateral loaded truck side protrusion and the height from ground level were measured, as well as the distance of the seat and the handlebars of the motorcycle from the ground. The comparison of all these data with the injury pattern of the body allowed the reconstruction of the chain of events that ended with the crash of the motorcycle against the loaded truck.

This case is unusual because of the devastating injuries of the body of motorcyclist involving the thorax and the abdomen. The autoptic examination compared with the engineering evaluation allowed the reconstruction of the dynamic of the crash, confirming that the transection of the body matched with the action of the loaded truck.

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### Motorcycle, Traffic Accidents, Forensic Sciences



## E1 The Detection of Latent Bloodstains Covered With Three Types of Current Top-Selling Paint/Primer Mixtures Using BlueStar®

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**Learning Overview:** After attending this presentation, attendees will better understand the effectiveness of using BlueStar® to detect latent bloodstains covered with up to seven layers of commonly sold paints available in retail stores throughout the United States.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by promoting a better understanding of the capabilities of BlueStar® in presumptively detecting the presence of human blood under one to seven layers of three commonly sold paint/primer combinations. Similar research has previously been completed; however, much of it is outdated and does not include paint/primer combinations commonly available at United States retail stores. Furthermore, most of the previous research involved the use of luminol, not BlueStar®, to detect blood.

Approximately .05mL of undiluted human blood was projected onto Gold Bond® ½" drywall samples in a manner to simulate impact spatter stains. The bloodstains were allowed to air dry for a minimum of 24 hours prior to painting. Four drywall samples were each painted with between one and seven layers of three of the most commonly sold paints within the United States: (1) Colorplace® Antique White Ultra interior paint plus primer; (2) Glidden® Warm Caramel interior paint plus primer; (3) Glidden® White Flat interior paint plus primer. Each layer of paint was allowed to air dry before the next layer was applied. BlueStar® was then applied, in total darkness, to the samples using a fine mist spray bottle. Results were photographed using a Nikon® D5200 Digital Single-Lens Reflex (DSLR) camera.

Chemiluminescence was observed upon application of BlueStar® in 83 of 84 samples. One of the Colorplace® Antique White samples covered with six layers of paint did not yield chemiluminescence upon application of BlueStar®. The intensity of the chemiluminescence was rated as medium or high in all samples with three or fewer coats of the paints. The intensity of the chemiluminescence gradually declined with the fourth, fifth, sixth, and seventh coats of paint.

Research has previously established the effectiveness of luminol in detecting latent blood that had been painted over; however, BlueStar® presumptive testing has been less studied. In addition, many of the previous studies resulted in inconsistent and variable results. Prior studies did not involve commonly sold paints currently available in retail stores and did not involve paint/primer combination mixtures. The focus of this study is to provide tangible results that relate to three of the top-selling, contemporary paint/primer mixtures sold by retail stores in the United States. Additional study recommendations include using BlueStar® to detect bloodstains painted over with additional layers of the paints used in this study, using diluted blood in a similar constructed study, and performing this study using different volumes of blood.

### BlueStar®, Blood, Paint



### **E2 The Visualization of 9mm and .40 Caliber Gunshot Residue (GSR) From Various Ranges of Fire With Alternate Light Sources (ALS) and Infrared (IR) Imaging**

*Mark Vecellio, MFS\*, Methodist University, Fayetteville, NC 28311; Sarah V. Morello\*, Raeford, NC 28376; Ryan Greaney\*, Spring Lake, NC 28390-2075*

**Learning Overview:** After attending this presentation, attendees will better understand the capabilities of ALS and IR imaging when viewing GSR on dark-colored fabrics.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by promoting a better understanding of the capabilities of, and differences between, ALS and IR in visualizing GSR on dark-colored fabrics.

GSR is composed of smoke resulting from combustion of propellant, metallic fragments from the bullet, unburned and partially burned gunpowder particles, and lubricants. GSR may be deposited on surfaces of objects at a crime scene or on clothing or skin of persons present during the weapon discharge. Visualization of GSR is an important task during preliminary phases of criminal investigations. Early presumptive detection can provide immediate insight for investigators by helping to establish if a bullet defect is an entry site, determining if a person may have handled a weapon or had been in close proximity to the weapon at the time of discharge, and in determining a broad, estimated range of fire.

GSR is easily observed on light-colored surfaces due to its inherent dark color. Dark surfaces, however, often obscure GSR. The two visualization methods chosen for this research include IR and ALS. Both of these methods can be practically employed by investigators during preliminary phases of investigations, including crime scene examinations to detect GSR on dark surfaces.

Magtech® .40 caliber 180 grain Full Metal Jacket (FMJ) and 9mm Luger® 115 grain FMJ ammunition were fired five times each from distances of 3 inches, 9 inches, and 18 inches into black, cotton T-shirt samples. Later observed were the samples using a Sirchie® TMX ALS (450nm) with orange barrier filters. Images were taken using a Nikon® D5200 camera and orange filter. A Fuji® XT1 IR camera with IR 830 and 850 filters was utilized, in conjunction with 100-watt incandescent light sources, to observe and photograph the samples with IR. For control purposes, all samples were viewed with the same ALS and IR equipment prior to shooting. No particles were detected on any of the samples prior to shooting.

GSR was visualized on all samples using both IR and ALS; however, differences were observed. ALS was more effective in identifying scattered particles while IR was more effective in identifying scorching, burning, and bullet wipe. The scorching and burning observed with IR was visible at the 3-inch range of fire for both types of ammunition, but not at 9- or 18-inch ranges. Bullet wipe was visible with IR for both types of ammunition at all ranges of fire. The scattered particles observed with ALS, as expected, formed increasingly tighter patterns as the range of fire decreased. Scorching, burning, and bullet wipe were not able to be detected with ALS.

The GSR observed with IR effectively revealed scorching, blackening, and bullet wipe in proximity to the bullet hole, allowing clear evidence of the bullet entry site, while the particles observed with ALS were scattered and more useful in generating a presumptive range of fire estimate. Any range of fire estimates derived from presumptive visualization, however, should not be considered scientifically reliable or conclusive, but can be helpful to investigators in early stages of shooting incidents.

The GSR identified with IR could be easily interpreted due to its close proximity to the bullet holes. The scattered particles detected with ALS, however, could potentially cause interpretation difficulties when other trace particles are present. Using IR and ALS in conjunction provides maximum benefit by allowing visualization of bullet wipe, burning, and scorching, along with the scattered particles.

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#### **Gunshot Residue, Alternate Light Source, Infrared Imaging**



## E3 Forensic Art—Know the Terms: Understanding Forensic Art Terminology

*Sandra R. Enslow, BA\*, Los Angeles County Sheriff's Department, Monterey Park, CA 91755; Daniel Marion, Jr., PhD\*, Denver, CO 80205-5630*

**Learning Overview:** After attending this presentation, attendees will understand the terminology used in forensic art. Facial reconstructions, composite drawings, age progressions, and video approximations are some of the examples that will be highlighted. This presentation will assist in understanding the complexities of the work performed by forensic artists and recognize the different uses for each application.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the definitions for each category of the forensic artist's work. In this presentation two General section forensic artists will present examples of casework they have handled and the definitions involved with each. These experienced forensic artists are collaborating to present this general knowledge to the Academy.

A confluence of facial anatomical sculpting ability and the data from a detailed forensic anthropological report are used in order to reconstruct the most reasonable likeness of the face the deceased wore in life. Facial reconstructions are cold cases. Given that the forensic artist begins with a skeletonized human skull, the following forensic anthropological terminology must be understood. Postmortem Interval (PMI), and its particulars, which include disarticulation (lost mandible or lost bones of the splanchnocranium), trauma (blunt force/sharp force, gunshot) and scavenging (animal gnawing) will be described. Any one of these or any combination of the above particulars can influence the outcome of a facial reconstruction. The presented 3D facial reconstruction was sculpted Fremont County, CO, by a member of the Colorado Coroners Association.

A combination of facial anatomical drawing ability and the data from a detailed forensic anthropological report were used in order to reconstruct the most reasonable likeness of the face of the deceased. A photographic image was used to develop a drawn 2D image of the face, rather than clay on the skull. This version of the 3D facial reconstruction is a faster process for getting the image to the investigators. As with 3D facial reconstructions, all 2D reconstructions are cold cases. PMI and its particulars, including disarticulation, trauma, and scavenging will be described. Any one of, or any combination of, the above particulars can influence the outcome of a facial reconstruction. The presented 2D facial reconstruction was drawn by the Los Angeles County Sheriff's Department lead forensic artist.

The composite drawing is a hand-drawn image. That drawing may be done with a pencil and paper, drawn on computer, or on a tablet with a stylus—any of these variations are composite drawings. Composites are traditionally developed using the cognitive interview process. Training is extensive for professional forensic artists in rendering the human face, understanding the anatomy, and interviewing witnesses. The presented composite was drawn by the lead forensic artist at the Los Angeles County Sheriff's Department for the Burbank Police Department, California. The description of the suspect was given by two different witnesses.

Forensic images are created from viewing video of persons of interest. Understanding the influence of light and shadow as well as Light Source is crucial in developing these approximations. Distortion, distance, and angle are three aspects that are taken into consideration in this process. These aspects assist investigators with a better understanding of who they are looking for.

Investigators, working on cold cases, often use an age progression. The image is developed using a combination of reference tools (early photos of the suspect, siblings, and parents) and knowledge of the Human Aging Process. Wrinkles, weight gain, and life style are other considerations taken into account.

Forensic art is a rapidly expanding discipline that requires specialized training, beyond advanced drawing skills. The forensic artist is called on for many different skill sets that assist in the identification process. These applications relate closely with those of other disciplines of the forensic sciences, especially forensic anthropology, forensic odontology, and forensic psychology.

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### Forensic Art, Facial Approximations, Facial Reconstructions



## E4 Extraction and Quantification of DNA From Buccal Cells of Peruvian Coca Leaf Users and Non-Users

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**Learning Overview:** After attending this presentation, attendees will be familiar with the practice of coca leaf use as a stimulant by a Peruvian population and the mechanism of studying the impact of coca leaf alkaloids on DNA samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the variability in DNA yields obtained from buccal samples and illustrating a study design to investigate the impact of alkaloids and other compounds on DNA yields from swabbing human subjects.

**Background and Objective:** The *Erythroxylon coca* plant, also known as the coca shrub, is a plant from the Andes mountain range in Western South America. Historically, Peruvian people have used coca leaves as a mild stimulant and some continue to use it today. Coca leaf users typically remove the stem or midrib from each leaf, fold the leaf in half twice, and place it in the cheek. Leaves are continuously added until a ball of plant material, approximately 2–3cm in diameter, is formed. The leaves are not masticated but moved around the cheek with the tongue while in contact with saliva for a period of time before being discarded. The primary alkaloid in the coca leaf is cocaine and the dried leaves contain approximately 0.6% cocaine; however, there are 18 other alkaloids present in the leaf, which may affect DNA yield from buccal cells or cause inhibition during DNA amplification. The purpose of this research was to collect buccal swabs from coca leaf users and non-users to determine if there are any differences in the quantity or quality of DNA recovered from the subjects.

**Experimental Design:** Buccal swab samples using 4N6FLOQSwabs™ Genetics were collected from 13 coca leaf users and 10 non-coca users in the village of Rosaspata in the Huamanga province of Peru after signing an informed consent form. A survey of the donors indicated that the age range for coca leaf users was 18 to 74 years. Three of the donors were male and ten were female. Three of the donors reported they use coca leaves once a day, four reported two or three times a month, and six said two to three times a week. Seven donors reported that they added a pinch of lime (CaCO<sub>3</sub>) to the cheek when using coca leaves. The number of leaves used ranged between 10 and 30 with an average of 17, and the length of time in the mouth ranged between 15 to 60 minutes with an average of 30 minutes. The non-coca leaf users included three males and seven females ranging in age from 14 to 44 years. All samples were extracted with the PrepFiler® Express™ Forensic DNA Extraction Kit. The Quantifiler® Trio DNA Quantification Kit was used for DNA sample quantitation and the GlobalFiler® DNA Amplification Kit was used for typing. The AB3500 Genetic Analyzer was used for Capillary Electrophoresis (CE) and the data files were analyzed using the GeneMapper® ID-X v 1.4 software (AT=100RFU).

**Results and Conclusions:** DNA samples were obtained from all 23 buccal swabs with observed differences in the quantitation values that can be attributed to, and expected from, normal variance in sample collection. No significant differences in the quantitation value means were observed between the 13 coca leaf users ( $M=8.98\text{ng}/\mu\text{l}$ ;  $SD \pm 4.199\text{ng}/\mu\text{l}$ ) and 10 non-coca users ( $M=47.39\text{ng}/\mu\text{l}$ ;  $SD \pm 23.69\text{ng}/\mu\text{l}$ ). The differences observed between the two groups did not reveal a statistical significance using the unpaired, two-sided *t*-test statistic ( $p=0.372$ ). All buccal swab samples yielded full DNA profiles with no apparent inhibition or degradation. All male subjects were coca leaf users ( $n=3$ ). No appreciable differences were observed in DNA yields from males versus females. Furthermore, DNA yields do not appear to be affected by the frequency, duration, or recency of cocoa leaf use. Based on the donor survey, the most recent coca leaf use was one day prior to the DNA sample collection ( $n=7$ ). It is possible that the lag in time of one day resulted in the metabolism of the alkaloids and/or the recovery of the mouth mucosa in which any impact on the DNA quantity and quality would have been attenuated to reveal any appreciable effect on the DNA sample.

### DNA Yield, Coca Leaf, Buccal Swab



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## E5 No Lab, No Problem! Practical Active Learning Ideas for a Forensic DNA Course

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**Learning Overview:** The goal of this presentation is to provide attendees with practical ideas for incorporating active learning into a forensic DNA course.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the benefits of active learning in the classroom, specifically the forensic science classroom. This presentation will dispel the myth that active learning in science can only be done through wet laboratory exercises. This presentation will emphasize that active learning in forensic science is not only a benefit, but it is essential for planting the seeds of success for our future forensic scientists.

This presentation will acquaint attendees with practical ideas for incorporating active learning into a forensic DNA course. These ideas can be modified for both undergraduate and graduate courses. Current education literature supports the active learning pedagogical technique, which is a technique that encourages students to take ownership of their learning through hands-on, interactive, and problem-based learning done both outside and inside the classroom. Theoretically, most instructors would agree that this type of learning is more beneficial for students in comparison to traditional lecture-style teaching; however, the problem lies in trying to figure out how to practically implement this in the classroom, especially when it comes to science courses.

In most science courses, active learning is typically done in the form of wet laboratory exercises. So what happens when the course does not have an associated laboratory? How can instructors implement active learning in science without one? These are questions many instructors struggle to answer. With much time spent already on course design and implementation, it can be difficult to find additional time to redesign a course to properly implement these active learning techniques.

The George Mason University Forensic Science Program aims to give students hands-on and interactive experiences in their forensic science courses both inside and outside traditional laboratory settings. Two of those courses are the undergraduate *Forensic DNA Sciences* course and the graduate *Survey of Forensic Chemistry, Biology, and DNA* course. Neither of these courses, at present, have a formal laboratory component; therefore, a mock case was added to these courses, which gives the students the experience of working a forensic DNA case from the moment it is received in the laboratory through the Combined DNA Index System (CODIS) upload. Student Assessment of Learning Gain (SALG) surveys were administered in both the fall and the spring semesters to evaluate the students' experiences with the mock case and how the active learning exercises impacted their experience in the course.

To aid in alleviating the difficulties many instructors face when attempting to implement active learning, attendees will be offered numerous examples that can be used in their own courses as well as a detailed explanation of the mock case exercises used in the forensic DNA courses presented.

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### Education, Active Learning, Forensic DNA





## E6 A Unique Case of Death by Electrocutation in Water in an Abandoned Building

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**Learning Overview:** The goal of this presentation is to provide the attendees with a case study that includes several variables that need to be taken into account in order to properly rule on a cause and manner of death while investigating a high-voltage electrocution in water death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing those involved in investigating electrocution deaths the tools and information necessary to provide a ruling on cause and manner of death.

Electrocution deaths are a rare occurrence in the field of death investigation and account for approximately 1,000 deaths in the United States per year.<sup>1</sup> The mechanism of death in electricity-related fatalities is most commonly ventricular dysrhythmia. The single most important factor to consider while investigating these deaths is the number of amperes involved, which is derived from the voltage and resistance of the electrical conduction system.<sup>2</sup> Oftentimes, high-voltage electrocutions result in readily apparent thermal injuries; however, no electrical burns or autopsy findings may be present to support electrocution in approximately one-half of low-voltage electrocutions, creating a diagnostic challenge for investigators and pathologists alike.<sup>3</sup> Additionally, in wet conditions, cutaneous burns can be rare because water provides an energization of the body that is too diffuse to cause focal electrothermal skin damage while also decreasing the resistance of the skin surface.<sup>2,4</sup>

The case presented here is a of a male found deceased in an abandoned building. The decedent was found by police lying in a pool of water in an electrical room. Initial assessment indicated the need for immediate inquiry to the local electrical company to assess for the presence of live electrical current provided to the building. The electrical company responded to the scene and reported a reading of 11,000 volts in two exposed wires. After the electricity had been safely disconnected, the scene was thoroughly investigated and photographed. Exposed wiring was noted in the room, along with tools indicating the decedent had been scrapping for material. A piece of black electrical tape was noted on the decedent's finger. In addition, paraphernalia consisting of two burnt glass pipes were found in his jacket pocket, suggestive of possible recent drug abuse. Apparent injuries noted to the body included lacerations of the forehead. At autopsy, additional minor injuries were noted, including a focal geographic, pale lesion of the right thumb, adjacent to the aforementioned electrical tape; samples of skin were taken for histological examination. Significant natural disease consisted of moderate cardiovascular and pulmonary disease. Results revealed microscopic characteristics consistent with electrothermal injury of the skin sample submitted; toxicological analysis was positive for cocaine and ethanol.

In electricity-related fatalities, a careful examination of the circumstantial data and a thorough scene investigation are essential. In this unique case, multiple alternative causes of death had to be considered due to the presence of illicit drug paraphernalia with positive toxicological results, significant natural disease, standing water (drowning), and external trauma. After thorough investigation and postmortem examination, the cause and manner of death was determined to be accidental high-voltage electrocution. This rare and unique case study reviews the key steps and investigative methods necessary to safely conduct a vital scene investigation and properly rule an electrocution death.

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### Electrocution, Water, Accidental



## E7 A Survey of Deaths in Judicial Control Areas in South Osaka

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**Learning Overview:** After attending this presentation, attendees will be informed about custody death cases that occur in Japan and prevention steps that could be done in the near future.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by stating the different types of death between the years in the south part of Osaka, Japan. From the legal medicine perspective, this presentation will show how to prevent the death of a suspect while in custody.

In Japan, when suspects or people in custody die in judicial control areas, specifically police stations, jails, or prisons, an autopsy is performed by a forensic doctor from the perspective of the responsibility of the officials holding them. The cause of death, mechanism of injury, and other matters are examined. Previous reports on cases of death in judicial control areas include cases of natural death, drug-related death, suicide, suffocation, and sudden cardiac death associated with violence toward or capturing of the suspect or person in custody by law enforcement, as well as death from violence by a cell mate in prison, although this is not directly related to law enforcement officers. From the perspective of legal medicine, however, no detailed investigations of deaths in judicial control areas have been reported, at least not in Japan. This study surveyed deaths in judicial control areas in the southern part of Osaka, the city with the second-largest population in Japan. This study examined the causes of death of suspects or people in custody, the possibility that the death could be foreseen, and the possibility that the resulting death could have been avoided. A legal medicine class analyzed cases of death within judicial control areas, including police stations, jails, and prisons, from among the forensic autopsies performed over the past 11 years (2008–2019). There were 1,705 cases of forensic autopsy and 22 deaths in the judicial control area. These 22 deaths included 14 deaths in prison, 4 in jails, 1 in a police station interrogation room, 1 in a police station holding cell, and 2 after persons found drunk on the street were held in a police station. Of these 22 people, 21 were male and 1 was female. Most of the cases were in their 40s, followed by their 70s.

The cause of death was violence in four cases, of which only one was a crime committed by a cell mate. The cause of death was suicide by hanging in ten cases, blunt force trauma in four cases (three of which were injury to the head), natural death in five cases (two cases of heart disease), drug poisoning death in two cases, and heatstroke in one case. Seventeen people were taken to the hospital and five were not. Of the 17 people taken to the hospital, 7 died at the hospital. The reason for not taking individuals to the hospital was that death was confirmed on the spot, evidenced by rigor mortis. Among those who were taken to the hospital, no one was taken to advanced treatment hospitals with more than 400 beds, six were taken to hospitals defined as general hospitals with about 300 beds.

The results of a survey of the causes of death by year showed that although many suicides by hanging were seen from 2008 to 2017, more acute drug poisoning deaths, natural deaths, and head injuries were seen in 2018 and 2019. These results indicate that in recent years, there has been a thorough awareness among overseers to prevent persons in custody from committing suicide. In 2018 and 2019, there were only cases of death of people held temporarily in police stations; no cases of death within prisons were confirmed. Thus, for these two years, this study surveyed only people held at police stations. Delays in taking individuals to the hospital, despite having a recognized injury, were due to judgments regarding the severity of an injury made by judicial control area overseers with no specialized knowledge. These judgments resulted in individuals being taken to the hospital only after their condition had deteriorated. If they had been taken to the hospital as soon as their injuries were discovered, it is possible that their survival could have been prolonged. From the results of this study, it is conjectured that while there is a declining trend in the number of suicides in judicial control areas, delays in dealing with recognized injuries may delay the possibility of anticipating a death and avoiding that result, ending with the person's death.

### Custody of Death, Judicial Control, Prison Death



## E8 Death of a Five-Year-Old Child in a Drum-Type Washing Machine: An Autopsy Case Report

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**Learning Overview:** The goal of this presentation is to explain the characteristics of children who die in drum-type washing machines according to postmortem imaging, blood gas analysis, biochemical examination, and pathological examination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by clarifying the characteristic pathophysiology of children who die after becoming trapped in a drum-type washing machine by various postmortem examinations.

The risk of children becoming trapped in the tubs of drum-type washing machines has long been noted in forums, such as the internet. However, few reports have examined cases in which children have actually died in washing machine tubs. An autopsy was performed in a case involving a child who died after entering the tub of a drum-type washing machine. This study examined the pathophysiology that resulted in death and similar previous reports and describes the findings.

The patient was a 5-year-old boy who was found in a state of cardiorespiratory arrest in the tub of a washing machine (diameter of opening, 60cm; depth, 29cm; washing machine tub volume, 82L) with the door closed. The boy was immediately transported to a hospital, where cardiopulmonary resuscitation was performed. However, cardiorespiratory function was not restored, and death was confirmed. Forensic autopsy was performed approximately two days after death.

The boy was 118cm tall and weighed 20.0kg (averages for a male Japanese 5-year-olds are 110cm and 19kg, respectively). Autopsy findings included severe facial congestion and petechiae of the facial skin and palpebral conjunctivae. Several organs exhibited congestion. Hemorrhagic spots were seen on the serous membranes of various organs, with particularly marked hemorrhagic spots apparent on the lungs. The heart contained fluid blood without soft clots. No findings indicative of marked trauma, intoxication, or hyperthermia were identified. These examination results suggested that asphyxia had occurred in this case. However, there were no findings indicative of cervical compression, oronasal obstruction, or the presence of a foreign body in the respiratory tract. Image analysis showed the child's body volume to be 19,329cm<sup>3</sup>, indicating that he would have been able to make postural changes inside the washing machine tub (82,000cm<sup>3</sup>). Consequently, impaired thoracic movement and postural asphyxia were considered unlikely to have occurred. Blood gas analysis showed no evidence of marked hypercapnia as compared with a group that had died from other causes.

In conclusion, the cause of death in this case was asphyxia due to hypoxia associated with being trapped in a closed space (i.e., a washing machine tub). Based on the respiratory volume of the child and the space in the washing machine tub, it was estimated that he became asphyxiated approximately one hour after entering the tub. To prevent such accidents, families should be informed of the possibility of accidents involving children when using a drum-type washing machine.

### Drum-Type Washing Machine, Asphyxia, Hypoxia

## E9 Parenting Behavior and Nutritional Deficits: Three Cases of Child Neglect

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**Learning Overview:** After attending this presentation, attendees will better understand the challenges of differential diagnosis of accidental and deliberate natures in some cases of malnutrition in children.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by understanding that prompt recognition of Failure To Thrive (FTT) in toddlers is essential to avoid negative effects on growth and long-term deficits in intellectual, social, and psychological functioning.

Neglect is a form of Child Abuse (CA) that also includes physical, sexual, and psychological abuse.<sup>1</sup> Child neglect is a rising but underreported problem in industrialized countries. Two main categories of neglect are described: deprivation-of-needs neglect (the caregiver's inability to provide for the child's basic needs) and supervisory neglect (failure of a caretaker to provide adequate supervision and safety for the child's developmental age).<sup>2</sup> Insufficient nutrition in children leads to FTT, clinically characterized by inadequate weight gain or growth, persistently falling below the fifth percentile for sex and corrected age of standard child growth charts.<sup>3</sup> Accurate investigations should be performed to rule out organic disease and ascertain nutritional deficits.<sup>4</sup> This form of neglect can lead to death by starvation in extreme cases.<sup>5</sup>

In order to contribute to the knowledge of the topic, this study describes three cases of various severity child neglect due to inadequate nutrition observed in the multidisciplinary "Bambi" Unit, dedicated to CA, of the Pediatric Hospital "Regina Margherita" in Turin, Italy. The involved children were all hospitalized with severe clinical symptoms and signs, including dehydration, muscle atrophy, wrinkled skin, and respiratory tract infection. All cases were referred to social services and reported to the judicial authorities.

Case 1 involved a 26-month-old baby girl sent to the Emergency Department by a general pediatrician because of impaired growth, multiple respiratory tract infections, and dermatitis. The infant weighed 9.64kg for a height of 83cm, less than the third percentile and between the third and the tenth percentile, respectively. Laboratory analysis detected iron deficiency anemia and excluded thyroid or celiac disease. Her mother exhibited severe parent-child interactional difficulties leading to inconsistent feeding patterns of the daughter.

Case 2, a 9-month-old baby boy, was admitted to the Pediatric Hospital with severe dehydration, hypotonia, muscular hypotrophy, and acrodermatitis. The infant weighed 6.02kg, less than the third percentile. Clinicians suspected milk protein allergy or malabsorption, but laboratory tests showed low levels of amino acids, cobalamin, and zinc as well as high ketones in the urine as signs of poor food intake. Moreover, spectroscopy showed delayed myelination due to a low level of cobalamin. During the hospitalization, a lack of supervision by his single mother was noticed as she was more interested in using her cell phone.

Case 3 involved a 17-month-old baby boy who was born at home without medical assistance and primary care; he had never received vaccinations. His mother weaned him with a vegan diet, resulting in the lack of many nutrients. In the past few months, he had developed suck-swallow incoordination, sialorrhea, anasarca, and walk and speech regression. At admission to the hospital, the infant weighed 7.70kg, less than the third percentile. Laboratory tests showed severe anemia, low proteins, undetectable cobalamin, and hyperhomocysteinemia. Magnetic resonance imaging of the brain showed diffused atrophy of the cortex; spectroscopy showed delayed myelination. A feeding nasogastric tube was placed with implementation of vitamins; speech therapy was required. Despite medical treatment, psychomotor delay persisted. The parents were worried about their child's health, and they carefully followed all the doctors' prescriptions in spite of their firm vegan and anti-vaccination beliefs.

This presentation provides attendees with a better knowledge of child neglect as one of the many causes of FTT. An early recognition of signs indicative of child neglect and abuse can be vital to prevent the situation from worsening, requiring prompt medical care and social support. Missed diagnoses of abuse and neglect because of carelessness or peculiar beliefs could put the child's life in danger.

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### Child Abuse, Neglect, Failure to Thrive



## E10 A Storm of Knives: Femicide and Attempted Suicide in an Ordinary Family

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**Learning Overview:** After attending this presentation, attendees will recognize the characteristics of a double crime scene dominated by multiple “white weapon” injuries. This case includes femicide and attempted suicide of a woman’s murderer husband.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing a multidisciplinary approach applied to crime scene investigations in a case of femicide and attempted suicide, following a love quarrel.

The term “femicide” defines an extreme act of violence against a woman perpetrated by a man that often occurs within a relationship or cohabitation. In this case, the weapon used was a kitchen knife. Knives are considered white weapons and are frequently used as sharp and/or sharp-point weapons; this kind of weapon produces typical wounds for their site and morphology. The characteristics and the extension of these wounds must be evaluated to differentiate homicidal from suicidal wounds.

On a cold winter night, two children, respectively eight and ten years old, frightened by the cries of their parents closed in the bedroom, went to the neighbor, who called the police. The police arrived in the house and found the master bedroom door closed from the inside. The policemen forced the door open and found the bodies of the two spouses, one over the other, on the floor. During the crime scene investigation, the forensic team found a knife block with two missing knives in the kitchen.

The lifeless body of the woman was inspected by the forensic pathologist: the wounds, all with clean and regular margins, were located on the face, neck, trunk, and upper limbs. Almost all the wounds were superficial: only two of them were broad and deep enough to cause death. The two injuries in the left hemithorax, at the autoptic examination, described two oblique intracorporeal traits parallel to each other. The first had the entrance wound located along the parasternal line between the second and third intercostal space, was directed from top to bottom and from right to left. This trait injured the left pectoralis major muscle, the underlying intercostal muscles, the pericardium, and the anterior and posterior walls of the right ventricle. The exit wound of the trait was on the back.

The second trait was located 8cm below and laterally to the first and involved the left ventricle of the heart. Below the woman’s body, her husband was surprisingly still alive despite the presence of a knife in the middle of his chest. The blade was the same model as those missing from the kitchen set. The man was taken to the nearest hospital, a chest Computed Tomography (CT) examination was performed with the *in situ* knife, then he underwent surgery. The hypothesized dynamics, based on the circumstantial evidence, were confirmed by the results of the autopsy performed on the woman and by data collected from the instrumental exams and body inspection conducted on the husband. Indeed, the presence of a deep wound with a knife in a highly vital area, such as the chest, in addition to the presence of multiple superficial stab wounds in self-attacking regions, such as wrists and elbow folds, with the parallel trend corresponding to test injury, suggested a suicide attempt by the man. The presence of wounds on the arms of the wife suggests a defense action; in literature, indeed, the defense wounds are common on the outer sides of the forearms, the backs of the hands, and the knuckles. The other typical knife defense wound is on the web between the base of the thumb and index finger when the blade is grabbed in an attempted pincer action, as in this case.

The presented case highlights and compares cutting wounds related to a homicidal event and a suicidal event, demonstrating how the two different diagnosis can be related by the circumstantial data; by the results of inspection; by the sites of the wounds, distinguishing between self-attacking areas or less; by the distribution and number of inflicted wounds; by the presence of defensive injuries (active and passive); or, conversely, by the presence of test wounds.

### Femicide, Knife Crime, Attempted Suicide



## E11 Fatal Dog Attacks: A Case Report and the Application of a New Forensic Approach

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**Learning Overview:** This presentation will impact the forensic science community by providing scientific data from a real case report of a Dog Bite-Related Fatality (DBRF), allowing discussion about a new forensic approach that could be applied to similar cases. Notably, the presented case demonstrated that the identification of a guilty dog through a buccal swab in the dog's mouth, while the most used methods to identify an offending dog are the application of forensic odontology and DNA analysis.

**Impact on the Forensic Science Community:** After attending this presentation, attendees will be able to apply a new forensic approach used to identify the offending dog when dog bite-related accidents occur. In light of this case report, the forensic community should consider using this approach in real casework studies with the goal of collecting new data, validating this technique for forensic use.

Statistics show an increase in the number of dog attacks; this phenomenon represents a hazard for the public health, both for the severe injuries and, in some cases, for fatalities. The identification of the guilty dog is necessary, considering the civil or criminal consequences for the animal's owner. Forensic investigations in dog attacks involve different pathological methods, most evaluating the canine Short Tandem Repeat (STR) typing in saliva traces on wounds, even if this technique cannot always be applied. The effort to perform new methods to identify guilty dogs represents a very interesting field for the forensic community. This case report aims to describe an interesting case report of DBRF, proposing an innovative approach based on the identification of the victim's profile in the dog's mouth by using a buccal swab on the suspected aggressor dog to find the victim's genetic profile.

This case concerns an 86-year-old woman who lived in a country house with her grandson. The grandson had a pit bull dog, who lived free in the garden with the aim of protecting their property. One day, at 5:00 p.m., the woman's nephew had gone away on several errands. At 6:00 p.m., the neighbors saw the old woman watering the garden. At 7:00 p.m., the nephew came back home; passing through the garden, he saw his grandmother on the ground with the dog eating her face. The grandson hunted the pit bull with a stick and called the police and rescue, even though the woman had died. When the police arrived, the dog had escaped, but he was found in a farmhouse near the woman's home about one hour later. The prosecutor alerted the forensic team.

At the external examination, the body of the woman was found lying on the left side, with arms outstretched. The woman's face was disfigured, which led to the exposure of the facial bones. The scalp was no longer present, leaving the skull exposed. The left arm was found partially disconnected, with exposure of muscles, tendons, and the humerus. The right forearm was damaged as well. The neck and upper portion of the chest had widespread linear wounds, compatible with scratches. In addition, roundish lesions were present on both her arms and neck.

After finding the dog, the veterinarian put him to sleep, allowing the collection of a buccal swab to the forensic examiner. This swab was performed approximately 1.5 hours after the assault. Another swab was performed on the victim's wounds with the goal of identifying the guilty dog. The dog's feces were not able to be used for forensic examination as the dog had escaped after the fatal attack.

The genetic analysis carried out on the dog's swab allowed identification of the victim's profile. Moreover, the dog's STR profile obtained by the swab performed on the victim's wounds was the same as the suspected dog. Finally, the veterinarian verified compatibility with the dog's dental arch and the roundish lesions found on the body. The autopsy ascertained the cause of death: the excision of the left brachial artery, with subsequent hemorrhagic shock, compatible with a dog attack, was described. In light of the genetic analysis, the dog's owner was accused of murder. Per research, this is the first time a new forensic approach was applied to DBRFs. Moreover, it was also confirmed by traditional forensic techniques.

### **Dog Bite-Related Fatalities (DBRFs), Buccal Swab, Forensic Dog Investigation**



## E12 Anabolic Androgenic Steroids (AASs) Use/Abuse: The First Italian Report

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**Learning Overview:** After attending this presentation, attendees will be familiar with the first Italian report about the dimension of AASs use/abuse. Notably, for the first time, this study was conducted on the data obtained through anonymous questionnaires to evaluate the voluptuary practices with a particular focus on AAS or smart drug use in Italy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the first data about the scope of AASs use/abuse in Italy, where it is illegal to sell AASs. Moreover, considering that the questionnaires map the voluptuary habits of the interviewees and their habits and perception about the use of “smart drugs” and “energy drinks,” a better comprehension highlights the way of AAS auto-administration, with or without other substances.

Even if AAS abuse is associated with a wide number of collateral effects, adolescents and athletes frequently use a large group of synthetic derivatives of testosterone, both for aesthetic uses and for improving performance. The market for performance-enhancing drugs is now huge and continuously increasing. The phenomenon of doping no longer affects only professional athletes but also subjects practicing sports activities at the amateur level. The adverse events for long-term usage among adolescents are very similar to those of adults, even if doses and duration are significantly less. On the other hand, acceleration of pubertal development and early epiphyseal closure, resulting in reduced adult height, are typical adolescent adverse effects; while masculinization of females and acne appears to be more severe in the adolescent.

This experimental study aims to realize the first map of the Italian phenomenon through anonymous questionnaires to evaluate the voluptuary practices linked to AAS or smart drug use in southern Italy. To achieve this goal, two questionnaires were administrated: Survey 1 was the Multidimensional Body-Self Relations Questionnaire (MBSRQ); Survey 2 was composed of 48 questions regarding the voluptuary habits of the interviewees about the use of smart drugs, energy drinks, and AAS, and the interactions with physicians regarding performance-enhancing drugs. Both questionnaires were administrated to a group of university students. Both surveys were performed between October 2018 and March 2019.

Two hundred forty-seven subjects correctly filled out the questionnaires; 48.2 % were male, 51.4% were female (age  $M=36.04 \pm 13.72$  years). All subjects enrolled were Italian and residents in southern Italy. The main results of the questionnaire demonstrated that eight people admitted the use of AASs (about 3.23%). Moreover, three people revealed they used them for more than 24 months. The motivations to use AASs are the same as described in the literature: they used them to improve their physical aspect and performance. Moreover, about 10% of the interview subjects admitted to the use of substances containing Tetrahydrocannabinol (THC). Interesting results are revealed about energy drink ingestion: about 50% usually used it to improve their mental activity. Furthermore, all subjects who admitted to the use of AASs have also consumed energy drinks and, in one case, THC. Comparison between these results and the results of the Section 1 test revealed that all AASs users had experienced previous body acceptance problems at some point in their personal history, which has now been resolved.

In conclusion, the questionnaire data demonstrated that the use of AASs in the young Italian population is about 4%; considering that in Italy, AAS sales are not allowed, online markets, the black market, gym friends, or physicians seem to be the first distributors of these substances. This aspect linked to the provenience of the AASs has to be recognized as very important both by the police and by the forensic examiner. Indeed, other unknown substances could be added to the anabolic agent, generating not only the adverse effects linked to the AASs use/abuse but also the organ damages linked to these unknown addicted substances. The last consideration is related to the combined use between AASs and energy drinks or THC: it is clear that the AASs adverse effects can be increased by their combined use.

### Anabolic Androgenic Steroids (AASs), AASs Italian Report, Energy Drink



## E13 Human Bones and the Estimation of the Postmortem Interval (PMI): An Experimental Study

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**Learning Overview:** After attending this presentation, attendees will have the results of an experimental study performed to define the PMI. Several authors have distinguished the forensic relevant postmortem interval between PMI <50 years and PMI >50 years. For these reasons, it is very important to define simple techniques that could be applied in every forensic laboratory to establish the PMI of human bones.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing experimental data of a study conducted in order to define the PMI on human bones. Several techniques were tested in this experimental study, and the histological and immunohistochemical examinations have shown a pivotal role in the determination of PMI.

Determining the PMI of human skeletal remains is a challenging part of the daily practice of forensic osteology. To clarify if further criminal investigations by law enforcement agencies are necessary, the PMI plays an important role. Skeletal remains can either be historical or recent. In a historical case, there will most likely be no interest in criminal investigations. On the contrary, further criminal investigations may be needed if human skeletal remains turn out to be recent. Therefore, it is of criminal interest to distinguish between historical and recent human skeletal remains by estimating their PMI.

The present study seeks to analyze the technical applications to define the best techniques to determine the PMI with simple techniques that could be applied in all forensic laboratories. All samples were selected analyzing documentations of all autopsies performed by the Institute of Legal Medicine of Foggia from 2001 to date (about 1,700 autopsies). Five cases of human bones were analyzed: in case 1, the bones were found in a natural ambient; after the forensic investigation, it was defined that they belonged to a subject who died ten years before the study. In case 2, the bones were buried in a glass box, considering that it was believed that they belonged to a saint's death in 232 AD; after the radiocarbon analysis it was established that they were dated between 200 and 400 AD. In case 3, the bones were found in a ravine 15 years after the death. In case 4, the bones were recovered under the natural soil; after the radiocarbon analysis, the bones were dated between 1300 and 1500 AD. Finally, in case 5, the bones were collected during the exhumation operation of a man had died and was buried 60 years before.

After the selection of the cases, all bones were worked in blind by the personnel of the forensic laboratory in order to define if each case was of forensic interest (PMI <50 years). Different non-invasive techniques were applied: a luminol test, an Ultraviolet (UV) -induced fluorescence test, histological tests (Hematoxylin-Eosin [H&E] and Masson's trichrome stains), and an immunohistochemical test (Glycophorin A antibody).

The results of the present study allowed the determination that only cases 1 and 3 are of forensic interest (PMI <50 years), while cases 2, 4, and 5 have a PMI >50 years. The differences are marked for all techniques comparing all cases, with an exception for case 5. Indeed, the PMI determination for this case was more complex: to define if it was of forensic interest, the application of the histological and immunohistochemical techniques were discriminant. Indeed, both the evaluation at luminol test and UV-induced fluorescence test were not able to define with certainty the PMI >50 years. In this particular case, to define the PMI, the histological and immunohistochemical examinations identified several changes in the bone cells that allowed the classification of the case as of non-forensic interest.

In conclusion, the results of the present study put in light the possibility of applying several non-invasive techniques to define, through the PMI, if the case can be considered of forensic or non-forensic interest. Moreover, the luminol test and UV-induced fluorescence test could be used as a preliminary investigation because their exclusive use is not always sufficient to define the correct PMI, while histological and immunohistochemical examinations could be very helpful for the forensic examiner.

### Skeletal Remains, Postmortem Interval (PMI), Immunohistochemical Stain



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## E14 Fatal Attraction: A Case Report of a Homicidal Drowning Involving a Couple

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**Learning Overview:** This presentation aims to explain a complete methodological approach, from the crime scene investigation to autopsy, and subsequent analysis (such as histological, immunohistochemical, and toxicological analysis) in a rare case of homicidal drowning. This case concerns a 32-year-old woman's homicidal asphyxia, due to drowning in a bathtub, after the loss of consciousness by manual strangulation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by examining a case of homicidal drowning in an adult, infrequent in the pathologist practice, showing the importance of using a complete forensic approach to solve the crime.

Homicidal drowning of adults is a rare event; according to the literature, this type of crime involves mainly children. This is due to the difficulty in realizing the homicide when the victim can defend herself. In these circumstances, the diagnosis of drowning is an important medicolegal issue. In cases of this type, it is possible to research the bone marrow diatoms. Their presence indicates that the decedent was breathing at the time of submersion and died due to drowning. Often the diatoms are helpful in the differentiation of drowning in a bathtub versus a naturally occurring body in water.

The following case concerns a homicide in which a double asphyxic mechanism was used: strangulation to stun the victim and drowning to kill her. A 37-year-old man went to the police station saying his girlfriend was dead in her apartment. The prosecutor's office organized an inspection of her house that was turned upside down. The corpse of the woman was discovered in a prone position in the bathtub, lying in a few centimeters of water. At the external examination, facial abrasions, petechial hemorrhaging in the conjunctivae, abundant froth exuding from the nostrils, and bruises on the forearms were found. An accurate inspection of the neck revealed little reddish abrasions in the anterior face and two little purplish bruises, of linear form, parallel to each other, on the left side of the neck. The suspicion of a struggle induced the forensic pathologist to perform fingernail swabs.

Simultaneously to medicolegal investigations, the prosecutor questioned the boyfriend of the woman. He confessed to having strangled his partner and then, to resuscitate her, placed the body under the water in the bathtub. When he realized that the woman was dead, he left the corpse there. The day after, an autopsy was performed showing scattered petechiae on the internal face of the scalp, on temporalis muscles and visceral pleura of both lungs and epicardium; hemorrhages of the left sternocleidomastoid and omohyoid muscles, of both sternothyroid muscles, right thyrohyoid muscle and hyoid bone; and red-tinged froth in the trachea.

Histological analyses showed scattered areas of contraction band necrosis in the myocardium, pulmonary emphysema, and alveolar edema. The immunohistochemical study performed on the neck muscles with anti-CD15, anti-tryptase, and anti-IL15 antibodies were positive, indicating the vitality of the lesions; the expression of HIF1- $\alpha$  on endothelial cells of lung vessels confirmed the asphyctic death. A DNA profile found on fingernails of the victim matched with the murderers, compatibly with the statements of the man.

In addition, a diatom test on femoral bone marrow was performed. The analysis of the sample using a phase-contrast microscope showed diatom frustules. This evidence represented the keystone to determine the manner of death: the fatal mechanism was attributed to drowning. By applying a methodological forensic approach, the actual dynamic of the event was reconstructed: the murderer knocked the victim unconscious by strangulation, then left the body in the bathtub, where the death occurred.

In complex criminal cases, in addition to the pathologist's work, the involvement of other figures is mandatory. Indeed, in this case, the contribution of the forensic pathologist was fundamental to identify the victim's marks on the murderer and vice versa; the ability of genetic investigations to place the murderer's DNA on the victim and the reverse; and the contribution of histopathological and immunohistochemical examinations to determine the exact cause of death. This case highlights the difficulty for a forensic pathologist to perform differential diagnosis between two mechanisms of death having overlapping pathological findings. The use of a rigorous forensic method in complex cases represents the essential tool for the forensic pathologist in identifying the true cause of death.

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### **Bathtub Drowning, Methodological Approach, Homicide**



## E15 An Evaluative Look at Shotguns: Pellet Spread

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**Learning Overview:** The objectives of this study were to determine: (1) if shotgun shells from different manufacturers would produce the same pellet count and pellet spread; (2) if those variables could be used to differentiate between 12-gauge and 20-gauge shotguns; (3) if pellet spread (impact area) correlates with firing distance; and (4) test the widely held concept that one inch of pellet spread is equal to one yard in muzzle-to-target firing distance.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing more accurate assessment of firing distance and observations of impact area as they relate to possible crime scenes where it is unknown what weapon and ammunition utilized. This could provide important investigation leads as well as corroborate any suspect or eyewitness statements.

At a firing range and target set-up, 12- and 20-gauge shotguns were used. The 12-gauge shotgun was an FNH SLP™ competition semiautomatic shotgun, full choke, 24-inch barrel. The 20-gauge shotgun was a Winchester® Model 12 pump-action, full choke, 24-inch barrel. These were used to test fire various brands of shotgun ammunition: Estate®, Remington®, and Seller-Bellot®. After firing 196 shells, the average values for pellet count, pellet spread area, and correlation with shotgun used and firing distances were analyzed for statistical significance ( $p < 0.05$ ) with an Analysis of Variance (ANOVA) and Regression Analysis tests.

The results indicated that firing distance and shotgun pellet spread were correlated. The data refuted the long-held notion that every inch of pellet spread is equal to a one-yard distance to the shooter. Additionally, it was determined that the pellet pattern alone could not be used to distinguish between the 12- and 20-gauge shotguns. The pellet count is different between gauges; but due to the amount of damage caused by close range shooting (<12 yards), is not an accurate assessment. No significant difference ( $p > 0.05$ ) was observed in the pattern between Estate®, Remington®, and Seller-Bellot® (00 buck). More importantly, when comparing firing distance and pellet spread, a significant difference was observed, ( $p < 0.0002$ ). Regression Analysis of spread at impact area vs. distance gave a  $R^2$  value of 0.97. This indicates a very strong and significant correlation ( $p = 0.01$ ). In conclusion, this study has provided a solid foundation to build upon in future research that will provide a new standard to accurately and quickly judge distance to shooter from pellet spread.

### Crime Scene Reconstruction, Forensic Ballistics, Shotguns



## E16 Examining Potential Degradation Between Antemortem and Postmortem Fingerprints

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**Learning Overview:** After attending this presentation, attendees will understand the influence of health-related conditions on the deterioration of fingerprints between antemortem and postmortem collection.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by clarifying the effect of death and various antemortem health conditions on the quality of biometric data between living and postmortem scans.

Various medical conditions and the early decomposition process cause both internal and external deterioration of the human body. If fingerprint deterioration occurs between the time of antemortem and postmortem print captures, the viability of a positive identification could be compromised. This project is a first step in understanding the correlations between medical conditions, fingerprint degradation, and biometric data quality. Through this research, law enforcement and other agencies will begin to understand how a subject's prior medical conditions can inform collection protocol used for biometric identification and facilitate decision making on the best methods for obtaining a positive identification.

Biometrics are measurable physiological characteristics that are used to classify both living and deceased individuals. Research has indicated that fingerprints are comparatively the most successful biometric indicator for positive identification. Therefore, in this study, fingerprints were used as the primary indicator to examine biometric degradation and quality data acquisition from an antemortem to a postmortem scan. For the purposes of this study, "quality data" refers to a threshold met by a ratio of numerical scores provided by two types of biometric scanning technologies. Fingerprint quality was determined by the National Institute of Standards and Technology (NIST) Fingerprint Image Quality (NFIQ) v.2 algorithm where prints are scored on a 1–5 scale, with 1–3 denoting adequate to excellent prints and 4–5 denoting poor quality prints.

Biometric scanning technologies have been used to capture all ten digits, where applicable, of 395 living predonors (individuals who have pre-registered to donate their bodies to the Forensic Anthropology Center). Of these 395 individuals, 15 are now deceased and had their postmortem images captured upon their intake into the Bass Body Donation program. For the purposes of this study, quality scores were relied upon to compare the antemortem captures to the companion postmortem images. Comparing the antemortem directly with initial postmortem capture determines if print degradation occurred between the living and deceased scans.

This study examines the correlation between certain mortality-inducing health conditions (e.g., cancers, diabetes, heart disease, pulmonary disease) and the time (in months) between the living scan and death with the change in quality of biometric data. In addition to the antemortem fingerprint images, Cause Of Death (COD) and date of death for the 15 individuals in this study were collected from the Forensic Anthropology Center's donation database. The goals of this study were to examine: (1) if any changes in fingerprint quality occur between the antemortem and postmortem captures; and (2) if so, could such changes be a result of known health conditions.

The preliminary results of this study show that fingerprint quality is generally consistent through end stages of life. Of the 40% of individuals ( $n=6$ ) who died of cancer, only one individual showed degradation in quality, while another individual had a slight improvement in quality. The other four individuals exhibited no change. Thirty percent of individuals ( $n=5$ ) died of heart-related conditions and two showed slight print degradation. All other postmortem prints remained of the same quality as their antemortem counterparts. The time between the antemortem scan and death similarly does not affect the quality of fingerprints. There was no change in the 30% ( $n=5$ ) of individuals that passed a year after their antemortem captures. However, two of the 30% that passed less than six months after their living scans showed slight degradation. Of the five individuals that passed seven months to one year after antemortem scans, two showed slight degradation.

This study has yet to show any apparent trends in the relationship between time between antemortem and postmortem scans or health-related conditions on the degradation of print quality between living and deceased print scans; however, analyses are ongoing. This study builds upon previous work and continues to support the utility of physiological biometric identifiers to obtain positive identifications in postmortem contexts. Postmortem biometric research has the potential to make important contributions to forensic anthropology and the law enforcement, military, and medicolegal communities.

### **Biometrics, Antemortem vs. Postmortem, Health Conditions**



### **E17 Training Needs for Search and Rescue (SAR) Teams During Mass-Disaster Fire Scene Recoveries: Lessons Learned From the 2018 California Camp Fire**

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**Learning Overview:** After attending this presentation, attendees will gain a better understanding of the training needs of SAR teams that are involved in large-scale fire scene recoveries. The lack of osteological training of SAR teams can hinder the search and recovery process of fatal fire victims, especially in large disaster zones. With this in mind, this presentation will provide insight on the key aspects of search and recovery that are most pertinent to SAR teams who lack training in fatal fire scenes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting a training guide that will be used to educate SAR teams and other recovery workers on the search and recovery of burned human remains in the context of mass-fatality fire scenes. This training guide will provide SAR teams with the basic tools to identify burned osseous material in the field, resulting in better outcomes for the search efforts, victim identification, and closure for families.

On November 8, 2018, the Camp Fire in northern California resulted in the destruction of 18,804 structures and 85 fatalities, making this the deadliest wildfire in California history. In large-scale disasters such as this, it is unfeasible for forensic anthropology teams to search every burned residence. For the Camp Fire recovery, many of the burned structures were first searched by SAR, California Department of Forestry and Fire Protection (CAL FIRE), and National Guard teams that lacked osteological knowledge, especially for burned remains. This disparity in knowledge concerning osteological identification and, more specifically, the appearance of bone within a burned context became evident early in the recovery process. SAR teams often requested short lessons on osteology and general tips for bone identification and human remains recovery in order to more confidently approach the large number of fire scenes. This research and its resulting product, a SAR fire scene recovery guide, will provide these individuals with the basic tools and knowledge needed to streamline search and recovery efforts for fatal fire victims.

The information within this SAR guide will address two main issues: first, where remains are most likely to be located within a structure fire, to streamline search efforts; and, second, how to distinguish human bone from materials that are commonly misidentified as human remains, including animal bones and burned debris. Data from the Camp Fire regarding the location of recovered remains will be used to extrapolate trends of high-priority areas within burned structures to be searched first. Tips on how to locate these areas within the debris will be outlined with accompanying photographs from prior house fire scenes. Furthermore, trends regarding materials commonly misidentified as human remains are detailed through professional experience from members of the California State University (CSU), Chico Human Identification Laboratory. Tips to aid in the determination of osseous from non-osseous material, and human remains from faunal remains, are provided through straightforward descriptions and archetypical pictures.

Ultimately, many individuals who participate in search and recovery efforts, including firefighters, police officers, and search and rescue personnel, would benefit from a training guide that outlines how to approach a scene and that serves to dispel common misconceptions surrounding house fire recoveries and the appearance of burned human remains. While this SAR guide provides information on how to approach a scene, it in no way serves to replace the role of forensic anthropologists, but rather to streamline the search and recovery process overall. Therefore, this guide will allow for a more expedient search and recovery process of fatal fire victims by providing SAR with tips and trends to more confidently approach mass disaster fire scenes.

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#### **Fire Scene, Search and Recovery, Human Remains**



## E18 Ice Cold Cases: When Glaciers Give Back Corpses

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**Learning Overview:** After attending this presentation, attendees will understand the complexity of personal identification on human remains recovered after a prolonged stay in a high-altitude environment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about the peculiar effects of glacier environment on corpses after a long period of time since death.

The Alps are the highest mountain system in Europe, with dozens of summits that reach over 4,000m and some massive glaciers. The region called “Valle d’Aosta” includes the Italian side of many legendary mountains, such as Mont Blanc, Monte Rosa, and Cervino. These mountains welcome countless hikers and skiers, and every year many people are involved in fatal accidents. While most of the corpses are recovered in a short time, in some cases, this is not possible due to the prohibitive environmental conditions that would put the rescue teams in serious danger. The fate of unrecovered bodies depends on where they lay. Glaciers are dynamic settings that significantly alter and gradually expose once-hidden corpses as part of their turnover process.<sup>1</sup> The glacier movement, which reaches speeds of about 100m per year, can affect where remains are ultimately recovered.

This presentation reports the data about the human remains of eight people returned by Alps glaciers from 1974 to 2018 at altitudes above 2,000m. They were recovered by the “Guardia di Finanza,” the military Italian enforcement that patrols the “Valle d’Aosta” summits, and were studied by the Aosta Office of the Medical Examiner. The aim of the identification effort after such a long time since death was to restore family links and clarify the fate of the missing people.

The recovered remains included bones and frozen soft tissues: in two cases, the entire bodies were recovered; in one case, bone fragments were not identifiable, while in the remaining five cases, a combination of numerous bones and teeth were collected. They were found in the hottest periods, following the melting of the glaciers. In all cases, the remains were accompanied by personal effects, such as clothing, technical equipment, or medicines. Personal identification required a holistic approach. In fact, achieving the goal was complicated by the large number and heterogeneous origin of the people missing in “Valle d’Aosta,” who were mostly tourists from all over the world.

The suspect’s identity was first tracked down through the careful examination of anthropometric characteristics and personal effects. The plausibility of the attribution was then assessed by estimating the trajectory that the corpse should have followed due to the glacier movements from the point where the person was last seen alive to the recovery location. Finally, DNA analysis was used as confirmation evidence, through the National DNA Database or if close relatives were available.

Six out of eight victims were identified: they were all adult male subjects (average age was 36.7 years) involved in accidents during excursions or skiing from 1954 to 1992. Two out of six were French, two were German, one was Turkish, and one was Italian. The average gap between the disappearance and the recovery of the corpse was 20 years. The average altitudes of the points of disappearance and of recovery were respectively 3,400m (range 4,000–3,000m) and 2675m (range 3,100–2,000m). The dynamics of the fatal accidents have been hypothesized as follows: two people were buried by avalanches; in two cases, trauma due to precipitation along crevasses was the cause of death; in one case, there was the combination of the previous two dynamics; and in one case, the victim froze to death during a snow storm. Bone analyses helped to distinguish peri-mortem from postmortem lesions and corroborated the hypothesis about death dynamics.

Factors such as cold temperatures, freezing, freeze-thaw cycles, and glacier movement are unique taphonomic agents.<sup>2</sup> Therefore, it is important to be familiar with these environmental agents and with their effects on bones. In fact, while glaciers have always been typically remote, in the foreseeable future, materials of anthropological interest may increasingly be revealed due to global warming and glacial retreat worldwide. This presentation provides attendees with a greater awareness of the importance of a multi-step approach to highly complex cases of personal identification on human remains that have been altered by extremely cold and ever-changing environments.

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### Human Remains, Personal Identification, Glaciers

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## E19 An Overview of 3D Printing in Forensic Science: The Tangible Third-Dimension

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**Learning Overview:** After attending this presentation, attendees will be informed of the current state of 3D printing in forensic science and learn about the benefits of incorporating tangible 3D reconstructions into the forensic science process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by advancing attendees' understanding of the potential applications and benefits of 3D printing reconstructions of forensic materials.

The goal of this research was to investigate the use of 3D printing across the forensic sciences and demonstrate examples in which 3D printing could feasibly be applied together with the useful and potential benefits to forensic science. Rapid development of 3D imaging and 3D printing technologies over the past decade have led to the increased affordability and availability of the techniques across health care and engineering industries. 3D-printed replicas provide a physical 3D object that users can hold, rotate, and inspect, facilitating greater haptic and spatial cognizance than with traditional 2D photographs or virtual 3D models.<sup>1</sup> These qualities make this technology ideal for demonstrating concepts around the inspection and interpretation of forensic materials. Further, 3D-printed reconstructions of forensic materials could provide a useful tool in many forensic science disciplines, such as crime scene analysis, intelligence gathering, and the presentation of evidence in court. While existing research has investigated the use of 3D replicas in areas such as medicine and anatomy, there is a distinct lack of published research investigating 3D printing across the forensic sciences.<sup>2,3</sup>

This research provides an overview of 3D printing in forensic science. An introduction into the different 3D printing technologies is followed by a review of the scientific literature and media publications to identify examples that have utilized 3D printing. A critical discussion of disciplines that may benefit from the inclusion of 3D printing reconstructions is put forward, including in forensic anthropology, forensic taphonomy, pattern and impression evidence, forensic archaeology, crime scene reconstructions, ballistic reconstructions, forensic engineering, forensic odontology, and facial reconstructions.

Further insights into the advantages and limitations of 3D printing technologies and printed replicas are given, including the potential effects of utilizing 3D prints as demonstrative evidence in courts of law. Examples of replicated “exhibits” and “scenes” that were 3D documented and 3D printed using Fused Deposition Modeling (FDM) are provided to illustrate several novel applications. These examples also show how 3D prints can be produced as scaled-up or scaled-down replicas, thus facilitating opportunities to exhibit objects at a manageable size, while providing an accurate, tangible reconstruction of the original material. This research demonstrated that despite the wide scope of 3D printing, this technology is underresearched and underreported in the forensic sciences. 3D printing reconstructions in forensic science can be affordable and attainable and deliver a tangible medium that can assist users with the interpretation and presentation of forensic materials.

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### 3D Printing, Forensic Science, Evidence Reconstruction



### E20 The Detection of Sodium Hypochlorite Adulterated Foods and Salsas in a Poisoning Investigation

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**Learning Overview:** After attending this presentation, attendees will better understand how to investigate a food adulteration in which bleach (sodium hypochlorite) was used as the adulterant. The goal of this presentation is to provide information to the scientist who may have to conduct future bleach-tampering investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing methodology that the scientist can use to detect and verify the presence of bleach and its breakdown products in difficult food matrices. This methodology can also be applied to other scenarios in which bleach has been used in a crime.

Two people were hospitalized after eating at two separate open food bars. Video footage at one of the food bars showed a man with a backpack putting an unknown substance on and in several food items. The man was later arrested by police and found to have a caustic bleach-containing product in his possession. This product was believed to have been used in the incident that caused injuries to the two people. Several samples of salsa and Chinese chicken entrees were analyzed by the United States Food and Drug Administration's (FDA's) Forensic Chemistry Center (FCC) and compared to the caustic bleach-containing product from the suspect. Bleach (sodium hypochlorite) is inexpensive, readily available, and found in numerous cleaning products. Household bleach contains between four to six percent sodium hypochlorite. Bleach is caustic, a strong oxidizer, and harmful if swallowed. The determination of the presence of bleach in foods can be difficult due to the instability of bleach in food matrices and interferences from the food with the detection of bleach.

The FCC has previously developed published methodology for detecting bleach (sodium hypochlorite) contamination in suspect beverages. Using these previously established protocols, seven suspect food items, one empty cup rinsed with a small amount of water for analysis, and a suspect adulterant (the caustic bleach-containing product) were characterized for the presence of bleach and bleach breakdown products. Characterization of the samples included the use of two oxidizing agent spot tests, iodometric titration, ion chromatographic analysis, headspace Gas Chromatography/Mass Spectrometry (GC/MS), and Liquid Chromatography (LC) /Charged Aerosol Detection (CAD). With the exception of LC/CAD, these techniques are routinely used at FCC to characterize samples suspected to have been adulterated with bleach or bleach-containing products. LC/CAD analysis has proven useful in providing a surfactant profile of cleaners. In this case, LC/CAD was used to identify surfactant characteristics of the suspect adulterant in comparison with the suspect food items.

Using the established protocol for the detection of adulteration involving bleach, all of the submitted food items were tested. The analyses showed that seven of the items and the caustic bleach-containing product were positive for the presence of oxidizing agents using the acidic diphenylamine and the potassium iodide starch test paper wet chemical tests for oxidizers. One of the food items had quantifiable levels of sodium hypochlorite still present. This food sample was analyzed using iodometric titration to determine its sodium hypochlorite content. Determinations were made as to whether the tested items contained active bleach, evidence to support the presence of bleach at one time, or no evidence to support the presence of bleach at any time.

#### **Bleach, Adulteration, Poisoning**



### **E21 Applying a Standardized and Scientific Approach to Recognize and Investigate Co-Occurring Criminal Forms of Fatal and Non-Fatal Asphyxiation in Order to Broaden Assessments to Include the Possibility of Other Types of Criminal Asphyxiation That Include at Least One Type of Aquatic Asphyxiation**

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**Learning Overview:** The goals of this presentation are to: (1) describe and list at least two types of criminal aquatic asphyxiation; (2) state at least three reasons why law enforcement, medical personnel, social workers, victim advocates, death investigators, and the jurisprudence community should be trained to be capable of identifying victims of fatal and non-fatal Co-Occurring Criminal Asphyxiation (COCA); (3) identify at least three approaches for continued improvement of identification and investigation of COCA; (4) list at least five questions that can be asked of potential victims of COCA; (5) provide at least four examples of what to look for, document, and collect on possible COCA crime scenes; (6) describe at least five injuries to look for and document when examining the bodies of possible victims of fatal or non-fatal COCA; and (7) list at least five current or past medical history diagnoses or symptoms that should be considered when investigating possible COCA incidents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the existence of COCA cases by presenting examples of fatal and non-fatal COCA case histories that include at least one form of aquatic asphyxiation. This presentation will impact the forensic science community by introducing practices to better identify multiple forms of criminal asphyxiation.

There have been significant improvements during the past two decades in the identification, investigation, documentation, research, prosecution, and prevention of Intimate Partner Violence (IPV) fatal and non-fatal strangulation cases.<sup>1,2</sup> Research, training, and tools, such as investigation forms for law enforcement and medical personnel, are continuing to make strides in overcoming challenges, such as the lack of victim self-reporting.<sup>3,4</sup> A next step is to broaden the net in order to do the same with additional types of criminal asphyxiation, such as manual suffocation, dunking, and waterboarding that occur to victims of IPV as well as additional populations, including pediatric, political prisoner, and trafficking victims.<sup>5-7</sup>

Once a possible strangulation assault has been identified, the investigation needs to continue looking for possible COCA that is defined as a criminal fatal or non-fatal assault involving more than one type of criminal asphyxiation. Some COCA incidents include a poorly understood type of asphyxiation that involves the use of water or other fluids to assault, torture, or kill victims. This study focuses on COCA incidences in which land asphyxiations (e.g., strangulation or manual suffocation) occur in conjunction with aquatic asphyxiation (e.g., dunking or waterboarding).

There are several challenges to recognizing cases of COCA, or a single-type of non-strangulation, fatal or non-fatal criminal asphyxia. It has been shown in several case histories that when effective strangulation investigation questioning results in an admission of non-fatal strangulation, those victims may not self-report that they also endured criminal aquatic asphyxiation. There have been cases in which children reported being the victims of non-fatal aquatic asphyxiation and were not initially believed so that timely investigations were not initiated. Although more law enforcement and medical investigators are becoming aware of the physical signs and symptoms of strangulation, this is not yet true for aquatic and other types of criminal asphyxiation.<sup>3,4,8</sup>

Fatal drowning is mainly a diagnosis of exclusion, making it challenging to recognize incidents of fatal criminal aquatic asphyxiation if the deaths are staged to appear as having different causes or manners of death, such as opioid overdoses or accidental drowning. Alternatively, there are cases of homicidal strangulation cases that were initially misdiagnosed as accidental drownings. In some locations, decedents who appear to have died from accidental drug overdoses or drownings may not have autopsy examinations.<sup>9</sup>

COCA incidents require evidence-based investigative questions specific to each type of asphyxiation as has been demonstrated by the need for specific assessment questions for the identification of strangulation. The first step to combating these crimes is creating an awareness of their existence within forensic, medical, and social work communities. The next step is the creation of effective, evidence-based, investigative questions and checklists that can be used by COCA-trained law enforcement, medical personnel, forensic interviewers, death investigators, and members of the jurisprudence community to identify cases and provide effective documentation of physical and psychological injuries and scene evidence that otherwise could be overlooked.

This study will explain the importance of applying a standardized scientific approach to the recognition and investigation of COCA. The forensic community will also be provided with suggested questions and checklist items that can be used as tools to help identify COCA victims. It is hoped that one result of this presentation is open discussions within medical and forensic communities to better identify, document, investigate, prosecute, and, most importantly, prevent COCA incidents.

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### Co-Occurring Criminal Asphyxiation, Strangulation, Drowning



## E22 An Evaluation of Various Swab Types for Recovery of Touch DNA From Firearms

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how new commercial products for the collection of touch DNA compare to traditional swab types for recovery of genetic material from firearms evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by evaluating both the adsorption and initial collection capabilities of the various swab types as well as the release and, thus, overall recovery of DNA based not only on swab substrate but shape, informing the practitioner community as to which swab types are best suited for the recovery of DNA from handled firearms evidence.

Amidst efforts to reduce illegal firearms and gun violence, laboratories have seen increased submissions of firearm-related evidence for genetic testing. Obtaining quality DNA profiles by following optimized protocols is instrumental in assisting with these criminal investigations. Published literature has established that the substrate of a swab (cotton, foam, nylon flocked, polyester, rayon, etc.) affects adsorption capacity and even DNA release, but more recent product designs have focused on the overall shape of the swab head to further enhance collection and concentrate touch DNA from challenging sample types, such as firearms. Currently, DNA collection from firearms is most often performed utilizing cotton- or nylon-flocked swabs that have rounded tips. Cotton swabs have an absorbent core that will draw samples to the center and potentially trap them. Nylon-flocked swabs have been developed to increase the amount of DNA released from swabs by having no internal absorbent core, keeping the sample on the surface for quick release.

This project focused on evaluating recently developed products by Gentueri, which include: SimpleSwab™, SimpleSwab2™, SimpleSwab3™, and SimpleSwab4™ that vary in material thickness and shape of the swab head. The efficacy of these new products will be compared to two commonly used swabs, including: general round cotton-tipped swabs and nylon 4N6FLOQSwabs™. Both the collection and recovery efficiencies of these swabs were evaluated. First, release of DNA from each swab was determined for a direct comparison between all swab types. Known concentrations of DNA were placed directly onto swabs to evaluate release and then again on a substrate and collected with each swab type to evaluate recovery and release combined. Each swab type was then used for the recovery of touch DNA from a firearm to simulate real casework samples. A double-swab technique was utilized for collection of touch DNA from four locations on each firearm, including the trigger/trigger guard, slide serrations, grip, and the magazine. Each sample will be separated into two halves and treated as two samples to determine variability among one swab. The swabs will undergo an organic extraction followed by quantification using Quantifiler® Trio, amplification using PowerPlex® Fusion 6C, separation on an ABI® 3500 Genetic Analyzer, and analysis using GeneMapper® IDX software.

A one-way Analysis Of Variance (ANOVA) statistic was performed for a quantitative comparison using resulting DNA concentration values. No significant difference was observed in DNA release from swab types tested with 125 picogram (pg) and 500pg total DNA [ $F(5)=3.11$ ,  $p=0.63$  and  $0.36$ , respectively]. Further work to evaluate additional DNA concentrations is currently in progress. Through quantitative and qualitative analysis of the DNA recovered, the optimum swab type/shape for the recovery and release of touch DNA from firearms evidence was determined. With the completion of this project, valuable information regarding the ability to increase genetic profile strength from front-end sampling of touch DNA will be disseminated.

### Firearms, Touch DNA, DNA Collection and Recovery



### E23 Validation of a Paper Analytical Device Through Analysis of Illicit Substances Found at Fatal Overdose Scenes

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**Learning Overview:** The goal of this presentation is to demonstrate the validation of a paper analytical device designed to identify various illicit substances with street samples collected from the scenes of overdose deaths. Attendees will leave the presentation with knowledge of the fabrication, chemistry, and testing ability of a new Paper-based Analytical Device (idPAD). The collaboration between a chemistry research lab, social scientists, and Coroner's office will also be discussed as a tool strengthen local communities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community by introducing an idPAD as a presumptive field test that can be used as an alternative or supplement to the current testing methods that can be used by law enforcement and first responders. This idPAD uses less solid than current presumptive tests, costs less than the test pouches, can be used in fewer than five minutes, and generates a complex color matrix to limit single color identifications. This presentation will lay out the different field samples and complex mixtures that were used to validate the device.

A 12-lane idPAD has been developed to detect and classify illicit substances, such as cocaine, heroin, and methamphetamine. The idPAD houses 12 different color tests that each target a different chemical functional group and react with a colored response. By running the tests simultaneously, a complex color "bar-code" is produced unique to each substance that can be read using an image library. The idPAD's ability to produce a unique color bar code for each substance, or mixture, presents a capability not currently seen in modern field presumptive tests. Initial testing of the idPAD with pure drug standards and fabricated mixtures demonstrated a 94% sensitivity and 100% specificity for cocaine, heroin, and methamphetamine in a lactose matrix. The limit of detection is well within the microgram range for all substances, and stability is consistent with current methods.

To validate the idPAD with collected street samples, a collaboration was started with the Marion County Coroner's Office in Indianapolis, IN. Many coroner's offices do not analyze the drugs found at the scene of the overdoses, relying only on toxicology data from the deceased. However, for this study, samples found at overdose scenes were collected and tested on the idPAD, with the results compared to Liquid Chromatography/Mass Spectrometry (LC/MS) of the drug samples and toxicology reports from the decedent. Trends on common drug mixtures as well as the highlights in differences between physical drugs found at scenes compared to in the toxicology report will be discussed.

#### Microfluidics, Paper Analytical Device, Presumptive Field Test



## E24 Success Rate Comparison of Latent Prints and Touch DNA From a Pistol

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**Learning Overview:** The goal of this presentation is to inform the community on the likelihood of obtaining prints of value from latent prints and/or obtaining a full DNA profile from touch DNA on a pistol.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by guiding and supporting decisions made by forensic scientists and investigators when processing evidence.

Firearms are a commonly shared evidence item between latent prints and DNA. This study determined the success rate of both disciplines regarding the processing of 80 pistols. The sample group was made up of 60 different pistols with a variety of 22 different brands. The samples were split into three groups: 20 pistols that were unclean with no documentation of their background that added a single contributor, 40 pistols that were cleaned and added a single contributor, and 20 samples that were cleaned and a variety of known contamination contributors were added before the main contributor to create a manufactured multiple source sample. Each main contributor had two minutes of handling for each pistol. The only requirements for the main contributor were to mock use the pistol twice, abstain from purposefully leaving prints or overloading with DNA, and handle the pistol in ways that could be reflective of prevalent handling. The handling was intentionally left open-ended in an attempt to have “normal” variation that could be seen in casework.

Using a wet double-swab technique, the DNA was collected from the grips and other textured surfaces that would not allow for prints of value. The pistols were then fumed in a cyanoacrylate chamber for latent prints. Using MBD, a fluorescent dye, and Alternate Light Source (ALS), it was expertly determined that from the 80 pistols, 11 contained prints of value, an overall success rate of 13.75%. This percentage is agreeable with the national average.<sup>1</sup> The DNA was amplified with GlobalFiler<sup>®</sup> chemistries and analyzed with GeneMapper<sup>®</sup> IDX. The analytical threshold was set at 50 Relative Fluorescence Units (RFU) and mainly spikes and off-ladder alleles were justifiably removed before collecting data. The touch DNA yielded full main contributor profiles for 73.75% of the samples. Of the remaining samples, 15% were missing only four or less alleles for the entire profile.

This study recorded if the alleles were present but did not expertly decide if each profile was of value. The target concentration was 0.4ng. The smallest target concentration to yield a full profile was 0.1035ng, which initially quantified at 0.0028ng/μL and had to be concentrated. There was not a noted correlation between obtaining a print of value and obtaining a full DNA profile, but the type of material used to manufacture the pistol could have affected the ability to leave a latent print or prevent leaving substantial DNA for testing. When there was a print of value, 63.63% of the time a full DNA profile was also observed. The degradation index was also calculated for each sample, and degradation was observed in multiple samples. The main factors for not obtaining a full DNA profile were degradation and insufficient DNA. This study could not incorporate every variation in casework, but serves as a foundation of what results could be obtained. This study is meant to guide investigators and forensic scientists to support their decisions for sending evidence to be processed by latent prints and/or DNA.

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### Touch DNA, Latent Prints, Firearm



## E25 Unique Dangers in the Medicolegal Death Investigation Process

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**Learning Overview:** After attending this presentation, attendees should be able to recognize potential dangers encountered during the medicolegal death investigation process. Attendees will also understand how, when unrecognized, these dangers could potentially lead to illness, injury, and even death of individuals involved in such investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the dangers involved in medicolegal investigations that may not be readily apparent. These unusual scenes generate concerns by law enforcement, medicolegal investigators, forensic technicians, and forensic pathologists because of the potential for illness, injury, or death.

Unrecognized, the unique dangers in the medicolegal death investigation process could potentially lead to illness, injury and even death of individuals involved in such investigations. This could include law enforcement, medicolegal death investigators, forensic autopsy technicians, pathologists, and threats to public health. Attendees will also understand how these types of dangers necessitate the utilization of a multidisciplinary medicolegal investigative approach to be able to identify and minimize or neutralize potential hazardous situations, elements, or environments.

The presentation of potential dangers in investigation, while not always foreseeable, often follow familiar patterns consistent with the suspected cause of death. Suicidal deaths involving the utilization of vehicle exhaust present the foreseeable risk of carbon monoxide poisoning within the investigation environment. Individuals dying from high-voltage electrocution present an environment that could be a risk for electrical shock. Persons dying from firearm deaths often present a scene where a loaded weapon is present and is an intimate part of the investigation. Conversely, death scenes often arise in which both the cause and manner of death are not immediately apparent, nor are potential hazards. This presentation describes multiple cases in which the dangers involved in the medicolegal investigation were not readily apparent. These unusual scenes generated concerns by law enforcement, medicolegal investigators, forensic technicians, and forensic pathologists because of the potential for illness, injury, or death.

**Case 1: Ricin**—Law enforcement and the coroner/medical examiner's office were summoned to the home of a 36-year-old Caucasian male. The death was reported as a possible suicidal overdose. At the scene, apparent drug paraphernalia was found as was a suicide note. After the initial investigation, the medicolegal death investigation found castor beans, acetone, and other materials that could be used for the production of ricin. This was communicated to law enforcement, who had observed the material but had not recognized this to be a potential threat. This led the medicolegal death investigator to discount her initial concerns and transport the decedent without taking further precautions.

**Case 2: Grenade**—Law enforcement and the coroner/medical examiner's office responded to a reported gunshot wound suicide that had occurred in a car. Upon arriving at the scene, the decedent was noted to have suffered an apparent perforating gunshot wound to the head. A semi-automatic handgun was in the vehicle in close proximity to the body. Photographic documentation was initiated and the firearm removed. During the examination, the body was leaned forward to visualize the back before removal from the vehicle. At that time, a safety pin from a hand grenade was heard, then visualized to have fallen to the ground. A grenade was then identified behind the back of the decedent. All personnel were evacuated and the bomb squad alerted. The grenade was removed and detonated by the bomb squad.

**Case 3: Firearm**—Law enforcement responded to the traffic crash deaths of two individuals attempting to elude police. The fleeing persons were driving at a high rate of speed within a business district. As they turned a corner, the vehicle struck a production class wheel loader. Both individuals were unidentified at the scene, and the vehicle was stolen. While the medicolegal death investigator was examining and photographically documenting the decedent, a cellular telephone began to ring from within the vehicle rubble. The medicolegal death investigator was asked to "grab the phone" before it stopped ringing. Upon picking up a pile of rubble, the medicolegal investigator felt something slip from the debris. He then observed a firearm falling to the pavement. It fell and stuck the pavement with the barrel pointing in the direction of the investigator. Inspection of the weapon revealed it to be loaded and ready to fire with the safety not engaged.

These usual cases required a multidisciplinary approach to facilitate and complete the medicolegal investigation process. These and additional cases illustrate the importance of remaining vigilant during the investigation process and how the utilization of a multidisciplinary approach may be necessary to assure the safety of all persons involved.

### Dangers, Multidisciplinary, Medicolegal



## E26 “Comet-Tailing” Associated With an Intermediate-Range Gunshot Entrance Wound

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**Learning Overview:** After attending this presentation, attendees will be more cognizant of the fact that, in certain instances involving susceptible ammunition, gunshot entrance wounds may display an associated “comet-tailing” pattern of vaporous lead/soot. This report will alert police, death investigators, forensic pathologists, and others of this rare but classic-appearing lesion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting a multiple gunshot wound homicide case in which one of the entrance wounds had associated “comet-tailing.”

Projectiles can be completely composed of lead or contain a lead core covered by a partial or full “jacket” of metal. Bullets are jacketed in part to prevent fouling of the weapon’s barrel while being fired. Metal bullet coverings can range from fully jacketed to “gilded.” Jacketed bullets have a relatively thick metal (usually copper, sometimes other metals) covering, while many .22 caliber rounds are “gilded” or “washed” with a copper-colored coating that appears to be sprayed onto the bullet surface. “Plated” or “electroplated” bullets can be considered “in-between” gilded and traditionally jacketed bullets. The metal covering of plated bullets is not as thick as the metal on classic jacketed bullets, but it is thicker than the light “wash” present on gilded bullets. Because this coating is thinner than classic jacket material, the thinner metal plating can, in some instances, fracture while being fired.<sup>1,2</sup> A homicidal gunshot wound demonstrating an unusual feature associated with a fractured plated bullet known as “comet-tailing” is presented.

A 23-year-old male was shot outside of his father’s house after visiting family. His wife, who was inside the residence at the time of the shooting, described hearing five to ten shots. The male was found by the driver’s side tire in front of his vehicle. Police and Emergency Medical Services (EMS) were called. Police found five 9mm casings and a fragmented bullet at the scene. EMS attempted resuscitation but was unsuccessful. At the medicolegal autopsy, the examination of the body was most significant for multiple gunshot wounds, predominantly of the head and neck. A perforating intermediate-range gunshot wound of the right side of the face had a very unusual feature, characterized by a dense area of soot-like material spiraling in a counter-clockwise direction, outward from the entrance defect, with a greatest dimension of approximately twice the diameter of the entrance wound. Although not previously described in the forensic pathology literature, the finding is nearly identical to what has been previously described in the firearms literature as “comet-tailing.”

“Comet-tailing” is an effect that can occur with certain bullets, usually those that are electroplated or plated.<sup>1</sup> This unusual effect occurs when a plated bullet’s electroplating material fractures upon firing.<sup>1,2</sup> Vaporous lead (presumably from the energy/heat caused by the firing of the projectile) escapes through the fracture site of the plating material.<sup>1</sup> Since the bullet is spinning along its longitudinal axis as it flies through the air, the vaporous lead stream spins as well. Thus, when the bullet enters the target, a spiral-shaped, so-called “comet tail” deposition of vaporous lead can appear on the surface surrounding the entrance site.<sup>2</sup> It has been suggested that in addition to the susceptibility of plated bullets to fracture when fired, features of the firearm itself, such as a flaw in the barrel, may predispose to fracturing of the electroplated covering of these bullets.<sup>1</sup> In the presented case, the entrance wound associated with “comet-tailing” was an intermediate-range shot, with an estimated distance of one to three feet. This case serves to alert police, death investigators, and forensic pathologists of this unusual feature that may be seen in certain cases of gunshot wounds wherein plated bullets fracture when fired from a weapon.

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### Comet-Tailing, Gunshot Wound, Forensic Pathology



## E27 Biomechanical Considerations in 3D Reconstruction of Shooting Events

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**Learning Overview:** After attending this presentation, attendees will learn how analysis of human motion can aid 3D reconstruction of shooting events.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how the accuracy and validity of 3D shooting reconstruction can be enhanced through surrogate studies.

3D computer modeling and animation software is an important tool in analyzing and illustrating shooting incidents because it allows us to accurately demonstrate the interaction between projectiles and objects in terms of time and space. Using 3D laser scanners, we can create highly accurate models of a scene. 3D models representing people, referred to as mannequins or character models, can be added to show the approximate body position of the person who was shot. Just as a pathologist places rods through a cadaver, lines can be added to depict bullet paths through the body of the mannequin.

One limitation of computer-generated mannequins is that they are not always able to replicate the body position or movements of a person. Computer models of people can be comprised of a series of articulated polygons or can be a mesh structure rigged with an internal bone system that deforms the mesh. In some situations, the mannequin will not accurately replicate how a real person bends, nor will it be limited to human range of motion. The mannequin's movements are controlled by the animator and may not accurately replicate human kinematics. Hollywood animators can create extremely complex and lifelike human models, which are controlled using motion capture data recorded from human actors. This level of sophistication with its associated cost is not often available to the forensic investigator.

In complex shooting events, it may be necessary analyze video of human subjects performing similar movements. Synchronized cameras can record surrogates from multiple angles as they attempt similar movements or body contortions. The video can then be analyzed frame-by-frame to derive motion data and body positioning, lending foundation to the accuracy of the reconstruction. A surrogate study may also reveal how surrogates approach a task or respond to stimuli.

This presentation will examine two officer-involved shooting incidents in which it was necessary to analyze the movement and positioning of surrogates performing certain tasks. In one case, an officer opened the door of her patrol car when she arrived at the scene of an alleged kidnapping attempt. A male suspect ran toward her car and jumped inside the vehicle on top of her. The officer fired two shots, striking the suspect in the shoulder and back. In analyzing the incident, surrogates were used to recreate the incident. A male surrogate ran toward a vehicle with an open car door and attempted to grab a female surrogate inside. Video analysis of the reenactment showed that when the male surrogate approached the vehicle, he slowed his pace and reached out with his right hand to brace himself against the B-pillar of the vehicle. He then dropped his left shoulder and led with his left arm as he ducked inside the open car door. This motion created the correct positioning for the shot angles to occur. The muzzle-to-target distances were consistent with stippling found on the body and blood spatter in the vehicle correlated with the location and orientation of the suspect's wounds.

In another case, an armed suspect was jumping over a backyard garden fence when he was shot by a police officer. The bullet entered the buttocks and traveled upward through the torso into the left chest. The bullet appeared to change direction, laterally, ending up in the left shoulder. There was what appeared to be an exit and reentry wound in the area of the left shoulder, suggesting the possibility that the suspect was shot while on the ground. As part of the analysis, surrogates were videotaped jumping over a similar fence. The surrogate study showed that there are numerous ways a person can approach and launch their body over a fence. However, going over headfirst, with one's left arm extended, created an alignment between the shoulder and the bullet path through the torso. It also created a crease in the skin that would explain the unusual shoulder wound.

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### Shooting Reconstruction, Biomechanics, 3D Modeling



## E28 Detection, Identification, and Characterization of Gunshot Residue (GSR) Using Raman Spectroscopy

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of recent advancements of the application of Raman spectroscopy for GSR analysis, identification, and discrimination. The implementation of advanced statistics to differentiate experimental Raman spectra collected from non-equivalent GSR samples will be discussed.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing the accuracy and effectiveness of shooting incident investigations.

Raman spectroscopy has numerous applications in forensic chemistry.<sup>2</sup> Raman spectroscopy is a technique that can obtain confirmatory class identification of analytes through low-intensity laser light scattering. The technique is non-destructive, rapid, sensitive, and requires little or no sample preparation. Raman spectroscopy offers several advantages over the current methodology for GSR analysis. The technique has been shown to detect components from both the organic and inorganic constituents of GSR on adhesive tape.<sup>3</sup> This is contrary to current GSR elemental analysis methods which rely solely on the detection of the heavy metals (lead, barium, and antimony).

Raman microspectroscopic mapping and multivariate analysis was recently utilized for the detection of GSR on adhesive tape. The study included a validation of the reproducibility/ruggedness and specificity of the approach.<sup>4</sup> Raman mapping for GSR detection was performed on an independent Raman microscope, not used to generate the training set. These independent spectra were classified against the original training dataset using Support Vector Machine Discriminant Analysis (SVM DA). The resulting classification rates of 100% illustrate the reproducibility of the technique, its independence upon a specific instrument, and provide an external validation for the approach. Additionally, the same procedure for GSR collection (tape lifting) was performed to collect samples from environmental sources, which could potentially provide false-positive assignments for current GSR analysis techniques. Thus, particles associated with automotive mechanics were collected. Automotive brake and tire materials are often composed of the heavy metals lead, barium, and antimony, which are the key elements targeted by current GSR detection technique. It was determined that Raman spectroscopic analysis was not susceptible to misclassifications from these samples.

Results from these validation experiments illustrate the great potential of Raman microspectroscopic mapping used with tape lifting as a viable complimentary tool to current methodologies for GSR detection. Furthermore, current methodologies are not well-developed for automated Organic GSR (OGSR) detection. A new two-step method for the detection and identification of OGSR was also developed.<sup>5</sup> This method utilizes highly sensitive fluorescence hyperspectral imaging of a sample area to detect potential GSR particles, followed by confirmatory identification of the detected particles using Raman microspectroscopy.

*This project was supported by an award from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.*

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2. Mistek, E., Fikiet M.A., Khandasammy S.R., Lednev I.K. Toward Locard's Exchange Principle: Recent developments in forensic trace evidence analysis. *Anal Chem*. 2019;91:637-654.
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4. Bueno J., Halamkova L., Rzhetskii A., Lednev I.K. Raman microspectroscopic mapping as a tool for detection of gunshot residue on adhesive tape. *Anal Bioanal Chem*. 2018;410:7295-7303.
5. Khandasammy S.R., Rzhetskii A., Lednev, I.K. A novel two-step method for the detection of organic gunshot residue for forensic purposes: Fast fluorescence imaging followed by Raman microspectroscopic identification. [under revision in *Anal Chem* 2019].

### Raman Spectroscopy, Gunshot Residue, Statistics





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### E29 A Serial Killer and Seven Homicides: Finding the Graves—A Multijurisdictional Investigation

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**Learning Overview:** The goal of this presentation is to highlight the methods used in multijurisdictional investigations involving local and state law enforcement, the Federal Bureau of Investigation (FBI), and the medical examiner's office to find the location, then recover a serial killer's burial site of seven victims.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the resources of local, state, and federal agencies to assist the medical examiner's office in uncovering mass graves and how this was achieved.

In 2007, scattered human bones were found on the ground behind a strip mall in Connecticut. At that time, it was suspected that the bones were from three individuals. Two skulls were recovered and later identified via dental radiographic comparisons. A third identification (by DNA) was made in 2014 from a few long bones that were recovered. These three individuals were from separate cities. Four local police departments had a possible suspect and compiled a list of missing persons, dates they went missing, and when they had been in contact with the suspect. Local police departments, working together with the department of corrections, state police, and the FBI, were able to track the suspect's movements and when he had been in the areas of the missing persons.

Once all the agencies concluded how many missing persons there were and if they could possibly be buried at the same location, the Connecticut Office of the Chief Medical Examiner was invited to the monthly Serial Killer Task Force meetings. This task force was comprised of investigators from local, state, and federal jurisdictions that convened once a month to share information and decide the next steps. A list of seven victims was compiled from evidence that was tested from the suspect's vehicle. In 2015, a comprehensive search of the 7.3-acre wooded area was performed using an FBI cadaver dog from Quantico, VA, a line search, soil probes, and ground-penetrating radar.

Based on the information obtained from the forensic analysis of the area, the anthropologist and medicolegal investigator focused on a 0.66-acre area. Once the smaller area was marked, back hoes were brought in, along with local, state, and federal personnel to assist with the excavation. After two weeks of searching and methodical test trenching excavations, five graves were uncovered, including the body of the victim whom the suspect was originally incarcerated for killing based on blood in his vehicle. The cooperation of multiple agencies across jurisdictions and the use of a variety of forensic tools helped determine the location of five grave sites where the remains of seven homicides were concealed (two were scattered on the surface and found in 2007). The suspect has since been tried for their deaths and found guilty.

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#### Forensic Search, Serial Killer, Excavation



## E30 Spicing Things Up: Death Due to the Use of the Synthetic Cannabinoid ADB-FUBINACA

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**Learning Overview:** After attending this presentation, attendees will be able to apply their understanding of thorough medicolegal death investigation practices to suspected Novel Psychoactive Substance (NPS) exposures. Attendees will be able to describe the elements of a multidisciplinary approach in NPS-related death investigations in order to facilitate effective communication and collaboration with law enforcement and medical, forensic, and public health providers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the literature regarding fatal synthetic cannabinoid exposure while underscoring the need for ongoing identification of these compounds in both postmortem specimens and drug paraphernalia.

Synthetic Cannabinoids (SC) are characterized as NPS and have been available for the past decade in the United States' illicit drug market. They are typically promoted as herbal preparations, branded as "not for human consumption," and sold in association with a variety of creative packaging and product names, such as "Spice," "K2," "Black Mamba," etc. Synthetic cannabinoids act as full agonists at the CB1 and CB2 receptors and as such they may be used as a substitute for cannabis.<sup>1-3</sup> Ever-shifting changes in NPS chemistry, manufacture, and distribution have continued to present challenges to the identification and interdiction of these compounds as well as the treatment of exposures and public health education strategies to combat their use.

This study presents the case of a 31-year-old male who was transported to an emergency department after he was found on the ground in a postictal state at a city park. A bystander witnessed him smoking an unknown substance in a glass pipe. He admitted to emergency medical services personnel that he had smoked "Spice" but was otherwise uncooperative. Clinical presentation included a severe tachycardia with an initial heart rate of 170 beats per minute and diaphoresis. He received symptomatic and supportive care and was discharged after approximately five hours of observation. Forty-eight hours later, friends found him unresponsive on the balcony of a house that had been rented for a bachelor party. He was pronounced dead at the scene. A black foil package labeled "Spanish Devil AKA Diablo" was found in his pants pocket, and a glass pipe was located near the body. Each item appeared to contain brown plant material.

In acute exposure, synthetic cannabinoids have been reported to produce nausea, agitation, tachycardia, hypertension, hyperthermia, delirium, seizures, renal failure, myocardial injury, and death.<sup>1-3</sup> Drug variability, dose, and potency as well as the presence of other licit or illicit substances may intensify the toxic effects of these compounds.

ADB-FUBINACA was identified in the postmortem peripheral blood specimen and in the drug paraphernalia. In 2018, Shanks et al. published the first case report of a death attributed to use of ADB-FUBINACA.<sup>3</sup> In 2019, this compound was among several synthetic cannabinoids to receive permanent Schedule I status by the United States Drug Enforcement Administration.<sup>4</sup> Structurally related to AB-FUBINACA, ADB-FUBINACA has a strong affinity for the CB1 receptor, which explains its clinical effects. Pathology, toxicology, and drug chemistry findings specific to this case will be presented in the context of previously published ADB-FUBINACA toxic exposures and fatalities. Based on the circumstances surrounding the victim's social history, clinical presentation, temporal relationship of SC use as well as the toxicology, drug analysis, and autopsy findings, the cause of death was attributed to ADB-FUBINACA intoxication. The manner of death was classified as accident.

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### Synthetic Cannabinoids, ADB-FUBINACA, Death



### E31 Collaboration in Action—A Case Study on Implementing the National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach Recommendations

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**Learning Overview:** After attending this presentation, attendees will understand the process from a Mountain West state in successfully implementing the *National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach* recommendations in the instructions, guidelines, and content in their statewide sexual assault kits.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by encouraging collaboration between forensic scientists, forensic medical/nursing examiners, and other multidisciplinary partners in implementing the *National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach* recommendations.

**Synopsis:** In 2013, Congress passed the Sexual Assault Forensic Evidence Reporting (SAFER) Act, which recommended the development of national best practices and protocols related to sexual assault kits.<sup>1</sup> The SAFER Working Group, comprised of multidisciplinary subject matter experts, was created to generate collaborative best practice guidelines. After multiple in-person and virtual meetings over two years, the United States Department of Justice released the SAFER Working Group's recommendations in the *National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach* in August 2017.<sup>2</sup>

Upon release of the *National Best Practices*, forensic scientists and forensic nurses in a Mountain West state (Utah) met to discuss implementation of the multidisciplinary SAFER Working Group recommendations. Specifically, the meetings focused on implementation of recommendations from Chapter 2: The Medical-Forensic Exam and Sexual Assault Evidence Collection. This presentation will explore the process of reviewing the sexual assault evidence collection recommendations in terms of the needs of the community and criminal justice system in Utah. Changes were made to the instructions, guidelines, and content of the sexual assault kits. This presentation will delve into specific changes made to the sexual assault kit content and guidelines, such as including masks for examiners in the sexual assault kits, no more collection of control swabs, and reducing swab numbers to focus on concentrating DNA material on swabs. Education was then disseminated to forensic scientists and forensic nursing and medical teams across the state on the changes and rationale for the changes.

The importance of a strong, collaborative relationship between forensic scientists and forensic nursing/medical examiners will be reinforced with suggestions on how to build local multidisciplinary teams to achieve best practices. Recommendations from *The National Best Practices* document were also used in recent Utah legislative bills regarding the submission and testing of all sexual assault kits and the creation of a tracking system with a victim portal. Thus, the *National Best Practices* document has positively influenced both practice and policy related to sexual assault in Utah.

#### Reference(s):

- <sup>1.</sup> *The Sexual Assault Forensic Evidence Reporting Act of 2013 (SAFER Act)*, P.I. 113-4, 1002, (o)(1).
- <sup>2.</sup> U.S. Department of Justice, Office of Justice Programs, National Institute of Justice, (2017). *The national best practices for sexual assault kits: A multidisciplinary approach*, <https://www.ncjrs.gov/pdffiles1/nij/250384.pdf>.

#### Sexual Assault Kits, Best Practices, Collaboration



## E32 Meningitis as a Cause of Death in a Medical Examiners Setting

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**Learning Overview:** After attending this presentation, attendees will understand the risk factors within the District of Columbia (DC) and the causes of fatal meningitis cases. This study will explore medical and social histories of each case.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by encouraging other medical examiner's offices to investigate the causes and specific risk factors of meningitis cases that occur in their jurisdictions. This is of great importance to decrease the likelihood of fatalities among patients diagnosed with bacterial or other forms of meningitis.

Meningitis is the inflammation of the meninges surrounding the brain and spinal cord.<sup>1</sup> The etiology of meningitis can be divided into bacterial, viral, parasitic, fungal, and non-infectious types. Each type can be caused by reasons such as injury, illness, substances, or chemicals. Viral meningitis is the most common form in the United States.<sup>1</sup> It is not as fatal as bacterial meningitis and has no specific treatment. It can be spread through bodily fluids, such as coughing, kissing, etc. Bacterial meningitis is primarily caused by a few different species of bacteria. It can be fatal and/or lead to brain damage. The population who is most at risk are patients with weakened immune systems and/or living in a college dorm or group home, where the exposure is high.

According to the Centers for Disease Control and Prevention (CDC), in the United States between 2003 and 2007 there were approximately 4,100 cases and 500 deaths due to bacterial meningitis each year.<sup>2</sup> Meningitis caused by the bacteria *Neisseria meningitidis* can be prevented with the MenB and MenACWY vaccines.<sup>3</sup> However, vaccine ignorance (lack of awareness about the vaccines) and vaccine hesitancy are on the rise. In fact, the World Health Organization cites vaccine hesitancy as one of the top ten threats to public health.<sup>3</sup>

This study analyzed autopsy, laboratory analysis, and investigative reports for significant medical history, signs/symptoms, residencies, and social history. This study looked over DC Office of the Chief Medical Examiner (OCME) cases over the past ten years (2008–2018). Overall, there were 22 cases of meningitis in DC. It was found that 55% were male and 45% were female. Decedents in this study were 68% Black, 22% White, and 9% Hispanic. The average age was 43 years old (the youngest was ~18 days old and the oldest was 70 years of age). It was found that bacterial meningitis was the most common cause of death in this study (91%), followed by viral (5%) and non-infectious meningitis (5%).

Interestingly, the 12 DC residents in this study were from every ward, averaging one to two decedents per ward. It was also discovered that decedents either had no medical history documented (22%), a history of strokes (27%), skull fractures/trauma (13%), and hypertension (27%). It was also found that 41% had a history of ethanol and crack cocaine abuse (22%) and used tobacco (27%). This is interesting because Phencyclidine (PCP) is one of the most abused drugs in DC, but was not present in any of these cases.

This research will provide statistical data for public health officials regarding the risk factors for meningitis. Given the amount of people that reside in the DC and the drug addiction rate, the risk of potential exposure is possible. This study shows that although some citizens in different wards have little to no access to major hospitals, this disease crosses all socioeconomic statuses. This study provides evidence of how important it is for medical examiner's offices to search for immunization records for each meningitis case, if accessible. Vaccines are available now and continuing education about these vaccines and meningitis could decrease the number of deaths per year.

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1. Understanding meningitis. *Meningitis Foundation of America*. 2017 Sep.
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3. Meningococcal meningitis. *World Health Organization*. 2019 Feb.

### Meningitis, Public Health, Death Investigation



### E33 Drug Abuse and Misuse Leading to Death in Tippecanoe County, Indiana (1993–2013): A Five- Year Update (2018)

*Matthew C. Wietbrock, BS\*, Lafayette, IN 47909*

**Learning Overview:** The goal of this presentation is to provide an five-year update to a previous report of drug abuse and misuse in Tippecanoe County, IN. In part, this will help gauge what, if any, impact recent awareness and prevention campaigns have had regarding the prevalence of drug- and alcohol-related deaths.

**Impact on the Forensic Science Community:** This presentation will benefit the forensic science community by focusing upon the current state of prescription drug abuse and by displaying the frequency with which such drugs are encountered in deaths investigated by the Tippecanoe County Coroner's Office. This will be accomplished by comparing a previous 20-year study to the past five years.

In 2014, the Tippecanoe County Coroner's Office conducted a comprehensive survey of all drug- and alcohol-related deaths for the previous 20 year period (1993–2013). The scope of this study was to evaluate what contributions prescription drugs, illegal drugs, and alcohol had played in the deaths of residents of Tippecanoe County, IN, from 1993 through 2013. This results of this study were discussed at the 2014 AAFS Annual Scientific Meeting, and widely reported around the local Tippecanoe County area. Five years have passed since this effort, and further work on this topic is eligible for discussion. The prevalence of mind-altering substances compared to all cases investigated by the Tippecanoe County Coroner's Office will also be discussed. This data will also be compared to national trends.

Tippecanoe County is located 68 miles northwest of Indianapolis, 124 miles southeast of Chicago, and is home to a population of 190,500. Tippecanoe County has a large manufacturing base. Caterpillar®, Inc., Subaru® in Indiana, Fairfield Manufacturing, and Wabash National® are all represented in the county. Tippecanoe County is also home to a major research university, which is still the area's biggest employer.

Drugs, like alcohol, which lower inhibitions or which can induce high-risk behavior, have long been recognized as being prevalent in cases where the manner of death is found to be accidental, suicide, or even homicide. Minimally, the presence of drugs, including alcohol, are investigated for regularly. Alcohol being readily available, and easily abused, can often be encountered in death investigations. Similarly, hard street drugs, such as cocaine, heroin, or synthetic drugs, have also been long known to play significant factors in death investigations. Use and availability of these drugs, especially heroin, have continued to rise in the North Central Indiana Region.

However, Indiana has experienced a meteoric rise in the use opioids such as methadone and oxycodone for pain management. The potential for chronic abuse of these prescriptions has become of rising concern for public health officials as is the potential for opioids to be combined with benzodiazepines and/or alcohol, which often lead to harming results. Nationally, these numbers have continued to rise, and Tippecanoe County is no exception. Several high-profile celebrity deaths on the national stage have added to the public awareness. The Centers for Disease Control report that overdose deaths related to the misuse of methadone have increased to account for one in three pain killer overdose deaths since 2009. The examination of 20 years of death statistics in Tippecanoe County yielded the frequency in which illegal, prescription drugs and/or alcohol were detected in decedents toxicological examinations, post mortem. This current study will provide updated data to consider.

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#### Drugs, Alcohol, Death



### E34 The Application of Virtual Reality in Training First Responders in the Proper Handling of the Dead

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**Learning Overview:** After attending this presentation, attendees will have a clearer understanding of the application of virtual reality as a training tool in the humanitarian forensic domain.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting: (1) the complexity of Management of the Dead (MotD) training for first responders; and (2) the need and importance of an experience-based, innovative, pragmatic approach of training modules.

The International Committee of the Red Cross (ICRC) has been assisting in humanitarian forensic response globally since the end of the 20<sup>th</sup> century; the nature of its assistance includes, but is not limited to, the training of first responders in the proper MotD in emergencies. Following disasters (natural or man-made), the role of the first responders in the search and recovery of the dead is crucial. Their swift, professional, and accurate actions can lead to a higher percentage of positive identifications if the correct information is collected at the early stages and adequately traced. Proper training for the shareholder agencies in the adequate response procedures is therefore essential. The challenges of the MotD training in different countries are extremely diverse, such as language limitations, differences in cultural, religious, and social needs, geographical and timely constraints, financial restrictions, changing jurisdiction of actors, the constant rotation of the acting personnel, etc.

Recently, education technology companies revolutionized teaching when they brought Virtual Reality (VR) technology to the classrooms. A 360-degree view of the content gives a more realistic feel to the trainees, while the interaction with VR content helps them explore the subject in more depth; the technology is not limited to any specific age group of students. In the MotD setting, the VR tool is also proving to be tremendously useful. The module does not require deploying heavy training material (mannequins, body recovery equipment, etc.); after launching the software, the trainees are immediately in the arranged and adjusted simulation. They can examine the environment in order to make decisions on their personal protection equipment necessities prior to going to the field to search for and recover disaster victims. The simulation can be modified regarding the stress situation (e.g., bombing, earthquake, tsunami) and the language can easily be adjusted. During the simulation, the trainer observes the trainee's advancement, as the whole simulation can be viewed on the computer or projected to a big screen; they can stop the process, give immediate feedback and recommendations. Moreover, the trainees can work in teams as other team members can, as well as follow what is happening inside the simulation—they can cooperate as they would on the field. Furthermore, the trainer does not have to be in the same room (or even location, town, or province) as the trainees; they can study the training process via computer connection, which is an added value in remote areas.

Applying the VR tool to MotD training does not intend to dismiss traditional training. On the contrary, it is intended to complement it. Ultimately, a better response is achievable via training following a disaster situation to avoid a high number of persons who remain unaccounted for and to return the missing to their families.

#### Humanitarian Forensics, Virtual Reality, Training



## E35 Examining the Impact of Trauma and Stress Across Forensic and Investigative Contexts

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**Learning Overview:** After attending this presentation, attendees will better understand contemporary literature and research with regard to trauma and stress in the world of forensic professionals, as well as the outcome variables subsequent to trauma exposure, which pose many risks for personal wellbeing.

**Impact on the Forensic Science Community:** The emotional and cognitive impact of trauma is of great concern for forensic professionals, who are at a significantly increased risk of exposure, but for whom there is a paucity of research understanding and even less on managing how individuals are impacted. This study seeks to elucidate these concerns as well as discuss a groundbreaking, comprehensive research study aimed at increasing not only our understanding of trauma in the forensic workforce, but the efficacy of trauma management.

While there has been a recent increase in attention to first responders and the impact of trauma (direct and vicarious), other investigative personnel or non-front line personnel are often overlooked or an afterthought in addressing these issues. It is important to understand and recognize how stress and trauma are tied to the forensic and investigative professions specifically, including in the form of vicarious trauma, and how exposure to trauma and stress impacts an employee's personal and professional performance. Raising the awareness of the various types of trauma that investigators and investigative personnel routinely encounter and the potential impacts this may have, not only on the individual, but on the investigation and/or service provision, is a critical next step in future-proofing the forensic and investigative workforce.

Paths forward and the development of Evidence-Based Policy (EBP) from related or tangential professions, such as police officers, dispatchers, firefighters, emergency medical services, trauma nurses, and other medical professionals may be adapted to the needs of various disciplines within the forensic and investigative workforce. However, a detailed understanding of unique and common stressors to those roles and responsibilities must be conducted to aid in the evaluation of existing policies and assistance typically available in agencies, if any, as well as to aid in empirically based policy development.

In seeking to serve communities as investigators or forensic professionals, many individuals are exposed to things that the average citizen cannot understand. These can include violent crime scenes, death investigations and autopsies, exposure risks, processing clandestine graves or receiving donated bodies for research purposes, reviewing materials in indecent images and child exploitation investigations, and so on, and sometimes these experiences stay with the individual. Recognizing that certain trends, such as the opioid crisis, can exacerbate the existing levels of stress and trauma faced by various personnel who often work in an interconnected environment, best practices suggest that an agency that is trauma-informed and has made preparations for employees will have better success in terms of keeping employees happy, healthy, and productive.

This presentation seeks to enhance the understanding of factors negatively impacting the emotional and cognitive impact of adverse experiences on forensic professionals. There is a paucity of research available with regard to resiliency, wellness, and burnout, and even less still addressing those for whom high-stress, violent, and traumatizing stimuli are not adverse risks, but job descriptions. These elements are endemic to the lives of first responders and forensic professionals, and yet extremely little is known about the long-term impact of chronic exposure to them, and the emotional, as well as cognitive, effect they may have.

This research seeks to evaluate a variety of factors via multiple avenues. The first is by identifying the prevalence of burnout in multiple forensic populations, including forensic anthropologists, sworn officers, medical examiners, and undercover operatives. Then cross-sectional analyses to scrutinize common and differential variables will be performed. Subsequently, participating agencies will have the opportunity to participate in an assessment of "trauma-informedness," with the opportunity for interventional recommendations. Finally, this ongoing research will use a targeted, brief neurocognitive assessment for individuals to evaluate the impact of burnout variables. Funding for this research is pending, but the current proposal is for a three-year study that is prepared to implemented at the end of FY2019–2020.

### Stress/Trauma, Burnout, Resilience

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### E36 The Effect of the Medicolegal Evaluation on Asylum Seekers: The Proposal for a New Operating Model

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**Learning Overview:** The goals of this presentation are: (1) to record the physical and mental consequents in migrant people (asylum seekers) that are victims of mistreatment and torture, distinguishing between consequents of mistreatments suffered in their own country, consequents of mistreatments suffered in Libya, scars of accidental injuries, and scars of tribal rites; (2) to focus attention on severe human rights violations in Libya, taking into account the data collection of medical visits performed; (3) to point out the need to increase the collaboration between forensic doctors, ethnopsychiatry experts, lawyers, and cultural mediators; (4) to show the collected data about correlation between medicolegal certificates and judgment of Italian territorial commissions; and (5) to present new operational proposals; that is, define new guidelines for torture assessment, starting from the Istanbul Protocol.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how sociopolitical conditions and processes shape the work of forensic scientists in this context, explaining the complexity of working in the current system, pointing out the need for a new approach to the evaluation of mistreatments and torture, going beyond the past protocols in order to respond to new sociopolitical needs, and explaining the importance of a new approach in light of the data that has been collected.

From May 2018 to July 2019, 93 asylum seekers were visited (most of them provided by local authorized community and health care units for immigrants). Approximately half of these were supported by social workers, psychologists, and lawyers who work in the extraordinary reception centers of the Sicilian island, while the approximately other half were sent by the Human Rights Legal Clinic of Palermo University. The asylum seekers were directed to medical-legal assessment and were subjected to a long audit that included the presence of the cultural mediator and the psychologist after they signed (informed consent) documents. The interview included a long anamnesis: family history (not only about health but also about relational life); schooling; past and present medical history (focusing on traumatic events, discriminating accidental events from abuse and/or torture). A separate section was dedicated to the abuse and torture suffered in Libya.

During the audit, subjects explain in detail: the means and methods that perpetrators used, the circumstances (location, for example, a prison/ghetto) and frequency; in this phase, the presence of the psychologist is very important in order to contain the risk of retraumatization. After the interview, the victims were subjected to objective structured clinical examination and photographic collection of scars. In relation to the needs of the case, radiological assessments (Magnetic Resonance Imaging [MRI], X-ray, ultrasound), specialized medical assessments (urology, otorhinolaryngology, dentistry, maxillofacial surgery, plastic surgery, neurology, gynecology) and non-medical assessments (anthropology and experts in sociology and transcultural psychology of gender identity) were requested. Researchers collected all the elements and wrote a report to send to the local governmental commission, while taking into account the Istanbul Protocol.<sup>1</sup>

Of the total number of asylum seekers assessed, 12 were women, 77 were men, and 11 were unaccompanied foreign minors. The countries of origin were mainly from the African continent: Nigeria, Niger, Senegal, Gambia, Libya, Guinea, Ghana, Ivory Coast, Somalia, Eritrea, Ethiopia, Sierra Leone, Benin, Cameroon, Liberia, Guinea Bissau, Guinea Conakry, and Bangladesh. Of the total number of asylum seekers, 32 have obtained international protection (12 by territorial commission; 20 by judges of the courts), in detail: 9 humanitarian protection, 1 subsidiary protection, 1 special case protection, 22 refugee status; of the remaining 54 subjects, 30 are awaiting the judgment of the court and there is no news on 28 because they were shifted in extraordinary reception centers in other regions of Italy. The data collected explain the need to prefer the medical-legal evaluation of the asylum seeker in a multidisciplinary context, not only in a medical context but also social and legal, pointing out the importance of the dialogue between forensic experts and lawyers to build a new protocol adapted to these new socio-political needs.

#### Reference(s):

<sup>1</sup>. United Nations. *Manual on effective investigation and documentation of torture and other cruel, inhuman or degrading treatment or punishment*. 1999.

#### Asylum Seekers, Torture, Abuse



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### E37 “V for Vendetta”: A Hidden Revenge Murder

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**Learning Overview:** The goal of this presentation is to demonstrate the relevance of a complete crime scene investigation and the meticulous postmortem analysis to define the differential diagnosis between suicidal or homicidal hanging.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by giving an useful example of the meticulous forensic approach to conduct during crime scene investigation and how it must be combined with a following multidisciplinary investigation.

In the literature, hanging has been reported principally as a suicidal modality or also as an accidental death. Homicidal hanging is exceptionally rare. The characteristics of ligature marks can be very important to distinguish between a suicidal or postmortem hanging but it cannot be the only nor the main element of discrimination. This presentation illustrates a failed attempt to hide a violent murder in a suicidal hanging, showing the importance of a complete and multidisciplinary forensic investigation.

A 48-year-old Tunisian laborer was found lifeless next to a farmhouse, suspended by two ropes wrapped around his neck at one end and anchored on a metal rod to the other, which was itself placed between two concrete poles. The victim’s head was covered by clothes. The local prosecutor requested the presence of the forensic team to perform a complete crime scene investigation. At the crime scene, policemen found a blood-stained stick in the farmhouse and drag marks on the ground around the house and next to the corpse.

The external examination was confusing: the body was covered by purple bruises, probably caused by an external blunt force trauma. Some typical signs of a suicidal hanging (such as the double ligature mark around the neck, swollen face, and protruding tongue) were also present. The Computed Tomography (CT) scan demonstrated multiple fractures of the facial bones and of the third cervical vertebra. At the autopsy, diffuse subarachnoid and intraventricular hemorrhages and multiple rib fractures were found. Tissues of the neck revealed diffuse hemorrhagic areas at subcutaneous tissues and at the left sternohyoid and mylohyoid muscles.

The histological examination, performed using Hematoxylin-Eosin (H&E) staining, revealed poly-visceral stasis in all samples; brain samples showed extensive subarachnoid and intraventricular hemorrhages and cytotoxic and vasogenic edema. The lung specimens demonstrated a massive pulmonary edema. The histological examination of ligature-marked skin and of cervical muscle samples revealed a massive presence of erythrocytes. The same skin samples were also studied with immunohistochemical technique using anti-CD15, anti-tryptase and anti-IL15 antibodies, that appear to be reliable parameters in the determination of ligature mark vitality. Results showed a dermal strong positivity to CD15, tryptase, and IL-15 in the marginal zones above and below the hanging marks. Toxicological examination revealed alcohol in the blood (154.91mg/dL).

According to these findings, the cause of death was attributed to homicidal hanging. In fact, despite multiple and massive blunt injuries on the head and thorax, the victim was alive at the time of hanging, because of the vital reaction of the ligature mark. Afterward, police identified the murderer, a Tunisian man, retired from the Foreign Legion. He revealed he had hit the victim with kicks and punches, then with several sticks and wooden shelves. At last, he hanged him to simulate a suicide. He then reported murdering the victim as revenge for all the mistreatment he had suffered by the victim during the period they passed together in the Foreign Legion. Indeed, the police discovered that the victim was a senior military officer in the Legion and the murderer was only a soldier.

In conclusion, the use of hanging to commit a homicide is very rare and a difficult interpretation finding for a forensic pathologist. In fact, the vitality of the ligature mark leads to a suspicion of suicide; generally, homicidal hanging was used in the past for executions, and it is really difficult to suspend a person against his will. In this case, it was possible to perform a murder because the victim was stunned by the alcohol and blows. The complete crime scene investigation conducted allowed finding the blunt instrument dirty with the victim’s blood, the drag marks of the corpse; combined with a complete postmortem investigation, this prevented forensic pathologists from making mistakes.

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#### Homicidal Hanging, Forensic Approach, Differential Diagnosis



## E38 Killer Tractor: A Forensic Methodological Approach in Work-Related Deaths

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**Learning Overview:** The aim of this study is to present a careful forensic methodological approach to be performed in cases of work-related deaths due to polytrauma. A case of death due to a tractor equipped with a mechanical cutter is hereby presented. Attendees will be shown how crime scene investigation elements and macroscopical findings were used to reconstruct the dynamics of the accident and to identify the cause of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the fundamental importance of combining a complete forensic methodological approach with crime scene investigation and a subsequent careful autoscopic examination in the case of work-related accidents with significant trauma. The autopsy should be based on the systematic evaluation of the vascular axes close to the lesions in order to highlight the source of the bleeding and attribute the correct cause of death. Given the proper cause of death, the association with the elements of the crime scene investigation allows the reconstruction of the accident dynamics with the final aim of highlighting critical issues in the security protocols.

One morning a 60-year-old man went to work on a farm where he had to mill olives after collecting them on a tractor with a mechanical cutter. Suddenly, the man got off the tractor and reached the front portion of it, getting sucked into the blades of the cutter. Shortly after, a colleague passing by noticed the man stuck in the blades and immediately called the ambulance. The medical staff quickly reached the accident site and positioned tourniquets at the lower limbs of the man, practicing a massive infusion therapy. Despite their efforts, the man died shortly after.

The crime scene investigation has highlighted that the tractor had the first gear engaged and the cutter indicator light turned on. Near the cutter and among its blades, multiple fragments of fabric similar to that of the victim's trousers were found in the proximity of a large pool of blood. At this point, the corpse was removed and a preliminary external inspection was carried out, confirming the presence of multiple and polymorphic lacerations located at various portions of the body, especially on the lower limbs. Particularly, two lesions appeared to be deeper, the first located at the upper third of the right thigh, the second located at the middle third of the left leg where it determined the nearly complete amputation of the lower third and the foot. For a complete definition of the cause of death, an autopsy was then ordered by the judicial authority.

The forensic investigation proceeded by single anatomical layers and highlighted the presence of areas of hemorrhagic appearance of the muscles underlying the abrasions and bruises. No traumatic lesions were found at the gross examination of the organs. The evaluation of the two deepest wounds was performed by proceeding equally to the dissection for single anatomical layers. The main vascular branches of the examined districts were then isolated. The evaluation of the left femoral artery and vein excluded the presence of laceration of the vessels, which were in any case characterized by widespread hemorrhagic appearance of the external walls. The evaluation of the left anterior and posterior tibial arteries and veins showed a complete laceration of both vessels. The histopathological examinations carried out later confirmed the generalized blood loss, therefore the cause of death was attributed to methemorrhagic shock due to the complete lesion of the main arteriovenous branches of the left leg. This data, together with the elements of the crime scene investigation, allowed the reconstruction of the dynamics of the accident, consisting of the victim's trousers getting caught in the blades of the cutter, which caused the death of the man.

### Polytrauma, Vascular Lesion, Work-Related Death



### E39 A Kindle Dinner With a Friend: Great Start, Bad Finish

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**Learning Overview:** The goal of this presentation is to highlight how the forensic pathologist needs advanced technology and the expertise of specialists in other forensic fields, without which solutions of crime scenes would not be found.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by underlining the importance of a multidisciplinary approach during a crime scene investigation.

From a review of the literature, it emerges how crime scene investigations give the best results when conducted by a multidisciplinary team. This case reports an example of how, during the crime scene investigation, the intervention of the police with only the forensic pathologist would not have been sufficient for the solution of the case. This case regards a Romanian man found dead in an abandoned cottage. Inside the cottage, several rooms were inhabited and in deplorable hygienic conditions. The corpse was found lying on the floor in a supine position at the center of one of these rooms. His clothes were dirty with blood. His head rested on a pillow also covered with blood. Other bloodstains were found on the sheets near the corpse and on the floor at the left of the victim. One of these stains reproduced a shoe sole and except for these stains, the messy room was clean of blood. The inspection of the other rooms was unremarkable, except for the last ambient where a plastic bowl, containing clothes immersed in a reddish liquid, was found. It was the scene of a murder, but nothing more than the body had been found. Thus, the prosecutor requested the intervention of the forensic team, with the K-9 dog unit and Obelux CR9 LED forensic lights. The team inspected all rooms, starting from the victim's body. This inspection revealed many biological liquid stains that, starting from the corpse, continued on the corridor walls and in the next room. In this room, the dog unit found two hidden wooden sticks, but when seen with CR9 forensic light, they turned out to be covered with biological liquid at one end. Clothes in the plastic bowl were also examined with CR9 forensic light and they too were covered with biological fluid, a sample of which was taken and analyzed by the geneticist.

The external examination partially confirmed the suspicion: the victim's chest and abdomen were covered by stamping bruises, which exactly matched in form and dimension the two wooden sticks. The entire body also presented extensive bruising and laceration due to hitting and kicking, especially to the face. The victim had multiple bilateral serial rib fractures and a pneumothorax. The final confirmation came thanks to the genetic investigation carried out on a suspect arrested a few days later by policemen. The forensic geneticist used swabs, both subungual and palmar, on suspect's hands. He also studied the bloodstain's DNA found on the suspect's trousers and even on the T-shirt found in the plastic bowl in the cottage. The genetic exams showed negative results of subungual swabs and positive palmar swabs for the victim's DNA. The biological stains on the trousers and T-shirt were blood from which was obtained mixed profiles linked to those of the victim and the suspect. The suspect, also a Romanian man, then revealed that he illegally lived in the cottage with the victim and that night after getting drunk together they started beating themselves until death. In conclusion, the current general tendency of the forensic community during crime scene investigations must necessarily hold a multidisciplinary approach, making use of technologies and collaborating with professional figures, every day, as this is indispensable for the correct solution of a case.

#### Crime Scene Investigation, K-9 Dog Unit, CR9 Forensic Light



### E40 Hanging Games: An Unusual Case of Accidental Hanging in a Child

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**Learning Overview:** The goal of this presentation is to show that in cases of hanging, immunohistochemical markers should be used in order to reach the diagnosis of vitality of the ligature marked skin.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing a rare case of accidental hanging of children and the use of new immunohistochemical markers to determine the vitality of the ligature mark.

Hanging is a form of ligature strangulation in which the force applied to the neck is derived from the gravitational forces of the body or a part of the body. Accidental hanging in childhood, although rare, has been reported worldwide. Hanging is an important cause of homicidal and suicidal damage in adults, but in children, it is usually accidental. According to available data, strangulation ranks fourth for causes of accidental deaths in children, following roadside accidents, drowning, and burns.

Some studies have shown that 80% of deaths by accidental hanging involved children under ten years old. The most common tools associated with these accidents are clothes or personal items, while young children explore the environment, unconscious of potential dangers. In cases of accidental hanging, it is more common to find an incomplete hanging; that is, when a part of the body is in contact with the ground. From the external examination, it is possible to identify the vitality characteristics of the ligature mark, such as small petechiae, serous vesicles, and hemorrhagic crests. During the autopsy, the hemorrhagic infiltration of the subcutaneous tissue and the neck muscles could be evident.

This case concerns a 9-year-old boy who was found hanged in the garage of his country house, partially suspended with the handle of a sack around his neck. According to the circumstantial data, the local prosecutor called the forensic team to investigate the crime scene in the suspect of *culpa in vigilando* of his parents. Indeed, the child was found unconscious, with his head in the handle of the sack, by his brother. The latter called their mother, who moved the son from the handle to try to resuscitate him, uselessly. During the crime scene investigation, the aforementioned sack was examined. This sack was on a wooden platform and had four blue jute handles. The handle where the mother found the child, according to the police data, was a distant 104cm from the upper edge of the platform below the sack. The platform had a height of 12.5cm.

The body of the boy was found in a sitting position, with the legs touching the ground. The child was 136cm tall. Ligature marks encircling the neck with bruises were described at the external examination. Other injuries were not present on the corpse, and only subconjunctival petechiae were described. Traumatic injuries were excluded after external examination and Computed Tomography (CT) scan investigation. The autopsy showed diffuse hemorrhagic infiltration of the neck muscles, pleural and pericardial petechiae, and marked pulmonary emphysema. No fractures of the hyoid bone or laryngeal cartilages were found. A routine histopathological study was performed using Hematoxylin-Eosin (H&E) staining. The microscopic observation of the brain specimens showed the presence of vasogenic and cytogenic edema; the lung specimens demonstrated massive endo-alveolar edema. The histological analysis of other organs was unremarkable. The skin specimens from the ligature mark presented the formation of intra-epidermal mild leukocytes reactions. Also, immunohistochemical investigation of skin samples was performed using antibodies anti-tryptase, fibronectin, TNFalpha, IL-6, IL-8, IL-10, MCP-1, IL15, IL1beta, CD45, CD4, CD3, CD8, CD68, CD20, CD15, and Langerhans cells to confirm the vitality of reactions in the ligature mark. The police investigation was oriented to an escape of the child from the house to the garage, probably for a joke. The prosecutor complied with this theory, supported by the forensic evidence of a rapid death by asphyxiation, and declared the parents innocent.

#### Accidental Hanging, Ligature Mark, Immunohistochemical Markers



### E41 The New Italian Law on Legitimate Defense: New Scenarios on the Crime Scene

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**Learning Overview:** The objective of this presentation is to analyze the innovative aspects of the new Italian law on legitimate defense approved in the Italian Senate on March 28, 2019, and entered into force beginning May 18, 2019.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by focusing on a much-discussed topic, the legitimate defense. Particular attention will be paid to the new Italian law and to the new changes and perspectives emerging in this legislative framework.

In the Italian legal system, self defense occurs if an individual is in imminent danger and must defend him or herself when no time is available to contact local authorities. Self defense should not be confused with revenge. The self defense institution is governed by Article 52 of the Criminal Code, entitled “legitimate defense” which reads: “It is not punishable who has committed the fact to have been forced by the need to defend a right of his own or others against the current danger of an unjust offense, provided that the defense is proportionate to the offense.” For a self defense instruction, there must be the sudden unjust onset of danger caused by unjustified aggression. The danger must be an immediate threat to either self or family. The amount of force used in self defense must be proportional to the aggression. The modification of Article 52 of the Italian Criminal Code extended self defense to include guests in another person’s home, a commercial residence, or in protection of personal belongings. It further allows the defender to use a weapon or other item to defend self or others.

The new Italian law, which consists of nine articles, incorporates traditional home defense and crimes against property. Article 1 of the law states that “there is always a proportional relationship” in cases of legitimate defense at home or in the workplace if someone “uses a legitimately held weapon or other suitable means to defend: a) their own or others’ safety; b) own property or that of others, when there is no resistance and there is danger of aggression.” Further, the law states, ‘he always acts in a state of legitimate defense who performs an act to reject the intrusion carried out with violence or threat of use of weapons or other means of physical coercion.’ Article 2 protects a person from penalty if they acted for the safety of themselves or others.

The law increases penalties for the offenses of home invasion theft, robbery, or housebreaking. The law excludes civil compensation for injury the home invader may have incurred, even if acquitted of the offenses in criminal proceedings. The law also allows for reimbursement or expenses for those acquitted based upon self defense. Finally, the law prioritizes the cases in the judicial system.

A recent example that used the new law was a recent argument between two neighbors, one of which murdered the other with a firearm. The assailant was acquitted because the decedent invaded the neighbor’s home and destroyed windows using a golf club. In this case, the courts found that the aggression and need for self defense were justified, in compliance with the new law.

#### Italian Law, Legitimate Defense, Crime Scene



## E42 Post-Coital DNA Recovery in Minority Proxy Couples

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the challenge faced in the timing of post-coital collections in extended intervals.

**Impact on the Forensic Science Community:** Combining the previous post-coital study data with the minority post-coital study, using enhanced Y-chromosomal Short Tandem Repeat (Y-STR) methods, DNA detection is possible for up to nine days in 65%–70% of reproductive-aged participants. Additionally, this study discovered a 20% increase in allele detection when swabs were combined. The recommendation is consideration for triage of delayed post-coital samples by forensic laboratories.

Minorities are less likely to report rapes. The Post Coital DNA Recovery (PCDR) study (2009–2014) subjects were White (93%) where expanded collection times were not generalizable to minority populations. Aims: (1) What is the time period for collection of post-coital DNA in minority women using Y-STR laboratory methods? and (2) when compared to the former study sample, what are the physiological conditions, factors, or activities in minority couples influencing post-coital DNA recovery? The design includes mixed-methods duplication perfected in the first study, embracing descriptive and inferential techniques. Aim 1 analysis used in PCDR in Minority Proxy Couples (PCDR-M) data only. Aim 2 combined data from both PCDR and PCDR-M studies. Combined, DNA recovery, a binary outcome accounting for repeated methods in population regression analysis, used Generalized Estimating Equation (GEE) methods.

To answer the first aim with PCDR-M data, studies data combined to test association of minority to non-minority allele detection and found there is no association using standard methods ( $p=0.6768, 0.9015, 0.4687, 0.7537$ ) or enhanced methods ( $p=0.3283, 0.4166, 0.5981, 0.3304$ ) across all times and locations, implying no allele difference between minority and non-minority populations. Fifty-three minority couples ( $N=106$ ) completed the complex PCDR protocol. The laboratory accepted 39 PCDR-M kits, but rejected 16 for allele levels, 1 for duplicate, and others for PCDR protocol non-adherence. Of the 23 (59%) kits accepted for study analysis, 19 had full and expected allele levels for baseline and post-coitus allele levels (4-, 7- or 9-days post-coitus) and 4 had 1 timepoint removed, resulting in 130 samples possible for analysis (46 4-days, 44 7-days, and 40 9-days). Of the 130 samples, the standard Y Filer® Plus method revealed 64 (48%) had at least one allele, and 66 (52%) had no detectable alleles. The enhanced Y Filer® method revealed 98 (75%) had at least one allele. Standard testing revealed 48% allele detection, and enhanced testing revealed 75% allele detection, duplicating the results from the first PCDR study and providing homogeneity necessary for comparison of the two data sets in Aim 2. To answer the second aim, data from study kits in the first PCDR study were combined with the PCDR-M study data for analysis, revealing the two populations were homogenous and met the inclusion criteria. The combined kits ( $N=89$ ) and potential 356 samples, 66 from PCDR and 23 from the PCDR-M, had full and expected allele levels for baseline and post-coitus allele levels (4-, 7-, or 9-days post-coitus). Standard methods revealed at least one allele detected at baseline (22.09%), 4-days (52.87%), 7-day (29.07%), and 9-day (33.33%). Conversely, standard methods revealed no alleles detected at baseline (77.91%), 4-days (47.13%), 7-day (70.93%), and 9-day (66.67%). Enhanced methods revealed at least one allele detected at baseline (63.64%), 4-days (91.01%), 7-day (78.41%), and 9-day (82.56%). Conversely, standard methods revealed no alleles detected at baseline (36.36%), 4-days (8.99%), 7-day (21.59%), and 9-day (17.44%).

The first PCDR study developed a validated *in vivo* study protocol and data model for establishing a valid scientific foundation for understanding extended interval post-coital DNA recovery and influencing variables of menses and hormone birth control use, which were duplicated with the PCDR-M study. Combined data also supports a single regional swabbing practice change—cervix and posterior fornix. Both study results validate collection from victims with expanded post-coital intervals, where laboratories use individual capacities and current science to determine which laboratory method is best suited for the submitted sample. The generalized findings to minority populations of victims increased the evidence for decisions made about DNA collection timing, recovery, and influences, with implications for improving laboratory capacities and algorithms for testing choices, minority victim engagement, and justice for the victim and accused alike.

### Post-Coital DNA, Y-STR, Rape



### E43 A Ten-Year Review of Opioid-Related Deaths at West Tennessee Regional Forensic Center: 2007–2017

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**Learning Overview:** The goal of this presentation is to analyze autopsy data from the West Tennessee Regional Forensic Center (WTRFC) from 2007 to 2017 to gain a better understanding of the effects of the opioid epidemic on West Tennessee and the surrounding areas.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying trends in race, age, gender, location, types of opioids, and drug combinations involved in opioid-related deaths in West Tennessee. Understanding the changing trends of opioid abuse is critical in order to predict the trajectory of the epidemic moving forward.

Prescription opioid deaths have tripled since 1999. Opioid overdoses kill 115 Americans per day on average.<sup>1</sup> Prior to 2014, prescription opioids were the primary driver of opioid-related mortality. In recent years, the United States has seen a steady decline in the rate of opioid prescriptions. At the same time, there has been a significant increase in the number of deaths attributed to non-prescription opioids, such as heroin, illicitly manufactured fentanyl, and fentanyl analogs. In 2017, among 70,237 drug overdose deaths nationally, 47,600 (67.8%) involved opioids, with increases across age groups, racial/ethnic groups, and county urbanization levels in multiple states.<sup>2</sup> The opioid epidemic is especially profound in Tennessee, which had the third-highest opioid prescription rate in the country in 2017 and an opioid-related death rate of 19.3 deaths per 100,000 persons, compared to the national average of 14.6.<sup>3</sup>

The WTRFC Death Investigation and Decedent Information database was searched for all deaths that listed opioid toxicity in the cause of death during the ten-year period from 2007 to 2017. Data gathered included age, sex, race, day, month, and year of the incident, location of death, manner of death, and cause of death.

Prescription opioids, such as methadone and oxycodone, were most common in the earlier half of the study period, but were quickly overshadowed by the rise in heroin beginning in 2012. The number of overdose cases involving fentanyl, most likely illicitly manufactured, rose precipitously beginning in 2015, spiking from 8 cases in 2014 to 51 in 2015, and peaking at 120 cases in 2017. Fentanyl analogs, such as acetyl, furanyl, and despropionyl fentanyl, began to appear as a cause of death in 2015 and 2016. Benzodiazepines were the most common class of drugs combined with opioids (46.3% of opioid-related deaths in 2016), followed by cocaine, ethanol, and amphetamines. Out of 1,344 cases of opioid-related deaths in 2007–2017, a total of 838 were male (62.4%) and 506 were female (37.6%); 1,108 were White (82.4%) and 231 were Black (17.2%). The most prevalent group across all ten years was White men, which comprised 50.8% of cases. Although Shelby County has a majority Black population, the White population was the most prevalent racial group involved in opioid-related deaths. In 2007–2010, the most prevalent age group involved in opioid-related deaths was ages 45–54. In the second half of the decade, people aged 25–34 were the most prevalent group.

In conclusion, illicitly manufactured opioids have become the new face of the opioid epidemic as opioid prescription rates have steadily declined across the country. Understanding the changing trends of opioid abuse is critical in order to predict the trajectory of the epidemic moving forward.

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#### Opioids, Overdose, Tennessee

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## E44 Epidemiological and Toxicological Profile of Homicide Victims in a Legal Medicine Unit in Brazil

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**Learning Overview:** After attending this presentation, attendees will understand the epidemiological and toxicological profile of homicide victims in Brazil.

**Impact on the Forensic Science Community:** This presentation will impact the forensic scientific community by highlighting what type of substance (legal or illegal in the context of a Brazilian legal medical unit) was found in the victim at the time of death. This presentation will also address the differences in substance types and the epidemiological profile of the victim. Finally, this presentation will study the seasonality of homicides and requests for drug testing.

Some studies point to an increase in homicide in Brazil since the end of 1970.<sup>1</sup> In recent years, there has been general stability in the homicide rates in Brazil, but considerable heterogeneity between the regions and various states of this continental country. The homicide rate in Brazil, per year, is 28.9:100,000 inhabitants, with gunshots being the most common cause (71.9%)<sup>1</sup>. Minas Gerais, the focus of this study, is a state located in Southeastern Brazil. It ranks as the second most populous state, third largest by Gross Domestic Product (GDP), and fourth largest by area. The state's capital and largest city, Belo Horizonte, is a major urban area and the sixth largest municipality in Brazil, but its metropolitan area is the third largest in Brazil with just over 5,500,000 inhabitants. The state has a homicide rate of 21.7:100,000, 71% by gunshot.<sup>2,3</sup> The use of illicit drugs is a risk factor for victimization by lethal violence and is frequently found in autopsies of homicide victims. This study aimed to evaluate the profile of these victims.

A cross-sectional retrospective study was performed on 1,382 homicide victims from 2014 for which toxicology had been performed in an Official Forensic Laboratory (Minas Gerais-Brazil). The results revealed a higher proportion of men ( $p=0.003$ ); black/brown skin ( $p < 0.001$ ); firearm history ( $p=0.007$ ); and a lower mean age ( $p < 0.001$ ); 943 homicide victims had a positive toxicology test; 72.2% of the positive tests were for cocaine or benzoylecgonine; 67.7% were positive for Tetrahydrocannabinol (THC) and/or its metabolite; and, finally, 43.1%, were positive for both cocaine and THC.

These findings indicated young Black or mixed race men have an increased risk of homicide. The role of illicit drugs in homicide goes far beyond intoxication as they are directly associated with age, skin color, and gender. These findings point to the importance of investing in social actions, public safety, and efforts to reduce drug use in this at-risk population.

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**Forensic Medicine, Forensic Toxicology, Homicides**





## E45 Characterizing Deaths Related to Hurricane Michael Using Vital Statistics Data

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**Learning Overview:** After attending this presentation, attendees will: (1) understand the process utilized by the Florida Department of Health (FDOH), Bureau of Epidemiology, to identify potential cases of death due to a natural disaster, such as a hurricane; and (2) recognize ways in which the partnership between public health and the death investigation community can be strengthened.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an example of collaborative interaction between the medical examiner system and death data in public health information systems, such as syndromic surveillance systems.

In October 2018, Hurricane Michael was the first Category 5 storm to make landfall in the contiguous United States since 1992.<sup>1</sup> This storm quickly developed and struck the panhandle of Florida. The storm resulted in 40 deaths in Florida, with a variety of manners of death and causes of death. These deaths were reported in the media for longer than three months post-storm.<sup>2</sup>

The FDOH is responsible for surveillance of events of public health significance. FDOH utilizes the Electronic Surveillance System for the Early Notification of Community-Based Epidemics (ESSENCE-FL) to monitor for events of public health significance and maintain situational awareness. ESSENCE-FL contains data from eight information sources. Data from Emergency Department (ED)/Urgent Care visits (UC), poison control calls, and death records are used in post-storm surveillance. ESSENCE-FL is one of the most robust syndromic surveillance systems in the country. These data are utilized for a number of public health program areas.

Before, during, and after Hurricane Michael, ESSENCE-FL data were leveraged daily to monitor visits at EDs, UCs, and Disaster Medical Assistance Team (DMAT) clinics. Post-storm electronic media reports of deaths attributed to the storm were tracked using Google® Alerts, which were monitored immediately post-storm through mid-March 2019 by FDOH to understand the health impacts of the storm. Death record data were utilized to review media-reported deaths related to Hurricane Michael as reported by the office of the medical examiner.

The media reported 40 deaths related to the storm; 8 were unable to be identified in the death record data. The decedents ranged in age from 24 to 94 years, with a median age of 66 years. Eight decedents were female and 32 were male. Thirty-four were white, and 6 were African American. Education level was reported as less than high school for 6 cases, high school or equivalent for 17 cases, some college or associate degree for 9 cases, bachelor's degree for 6 cases, 1 case had a master's degree, and 1 case's education status was unknown. Most decedents were Florida residents, but four decedents were residents of other states. The Manners Of Death (MODs) for these reported deaths were accident (19), natural (17), suicide (2), and homicide (2). Autopsies were performed on 23 of the 40 cases.

There was a higher incidence of deaths (58%) in those who had lower education levels (i.e., high school or less). Those with lower levels of education typically have a lower income, which impacts their ability to prepare or evacuate for a coming storm.<sup>3</sup> This suggests an opportunity to improve public health messaging and support for those with a lower education status. In addition to providing some of the details of the deaths, this report will highlight areas of intersection between public health data and medical examiner/death investigation information.

This event illustrates the importance of collaboration and coordination between medical examiners/death investigators and public health practitioners. Increased collaboration in the future via electronic record data sharing would improve the timeliness of reporting death data of significance. This will result in decreased delays in public health interventions immediately following a disaster. Syndromic surveillance systems like ESSENCE-FL are able to facilitate near real-time data sharing and allow better access to records for other public health use purposes, such as opioid overdose fatalities and injury prevention.

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### Disaster, Death, Surveillance

## E46 Modeling Postmortem Submersion Interval (PMSI) Estimation From the Microbiome of Bone in a Freshwater Lake

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**Learning Overview:** After attending this presentation, attendees will understand how microbial community changes across Accumulated Degree Days (ADD) can be used to aid medicolegal death investigators and forensic pathologists when skeletal remains are recovered from freshwater lake environments.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing proof of concept for the use of microbial succession on skeletal remains submerged in a freshwater lake to predict the long-term PMSI.

Understanding decomposition and its associated factors is imperative to aid medicolegal death investigators and forensic pathologists in estimating the Postmortem Interval (PMI) on land or the PMSI in water. Because human remains deposited in aquatic environments are exposed to conditions distinct from those of terrestrial environments (i.e., water salinity, temperature, depth, tides, currents, pH, aquatic scavengers, floating or sunken debris, oxygenation, etc.), traditional PMI methods (i.e., invertebrate succession) are not transferable. With advancements in next-generation sequencing technology, improved bioinformatic pipelines, and the identification of changing microbial communities in animal models, recent studies have sought to utilize bacterial succession as a method for estimating PMSI. While initial studies provide a foundation for understanding microbial communities in aquatic decomposition, their applicability to humans is limited due to the fundamental differences between aquatic (e.g., salmon) and terrestrial organisms.<sup>1</sup> In addition, studies performed with porcine remains have not focused on long-term PMSI estimation extending to the skeletal stages.<sup>2,3</sup>

Fresh pig (*Sus scrofa*) bones ( $N=100$  rib and  $N=100$  scapula samples) were obtained from a butcher. Bones were placed in cages attached to a flotation device and submerged in Henley's Lake, White Hall, VA, from November 2016 to June 2018. Water temperature and environmental parameters (i.e., pH, Dissolved Oxygen [DO], salinity, etc.) were recorded using waterproof loggers and a YSI® Sonde, respectively. Every 250 ADD, five scapulae, five ribs, and 500ml of water were collected and stored at either -80°C or 4°C until processed. Water samples were filtered on 0.22µm filters. Bone samples were cut and ground into a powder using liquid nitrogen in a mortar and pestle. DNA from filters and powder was extracted and purified using ChargeSwitch® gDNA Plant Kit and DNeasy® PowerClean Pro Cleanup Kit, if necessary. Following the protocol established by Kozich et al., sequencing-by-synthesis of microbial 16S recombinant DNA (rDNA) variable region 4 was performed via Illumina's® MiSeq® 2X300 paired-end sequencing.<sup>4</sup> Data analysis and visualization was completed via the MiSeq® mothur SOP, mothur version 1.35.9, and R studio.<sup>5</sup>

Preliminary analyses indicate significant differences in bacterial communities among sample types (i.e., rib-water-scapula). For rib and scapula samples, phylum-level relative abundances differed significantly across ADD. Similarly, beta-diversity (Bray-Curtis) successional patterns were visible in ordinated space for both sample types. Spatial ordination was correlated with environmental parameters and ADD. Additionally, bacterial communities identified on scapula and rib samples differed significantly with ADD. With regard to alpha diversity (shannon), rib and scapula samples demonstrated a curvilinear relationship with ADD; therefore, fifth and fourth polynomial regression models were applied, respectively. Based on these results, bacterial succession patterns were used to develop a long-term PMSI estimation model.

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### PMSI, Freshwater Lake Bone, 16S rRNA Gene



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### E47 Entrance and Exit Hole Characteristics: Bullet Types, Substrates, and Firing Distance

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**Learning Overview:** The goals of this study focused on the ammunition, the target substrate, comparing the shape of the entrance and exit holes, and the diameter of the entrance bullet hole from two firing distances.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by investigating the correlation between class characteristics of bullets and the diameter of entry and exit bullet holes in different substrates. This presentation will also help the forensic science community by providing guidance for further firearms research.

This study was designed to compare the shape and diameter of bullet entrance holes fired from two different distances. The comparison concentrated on ammunition and target substrate. A Smith and Wesson® SD9VE 9mm Luger® firearm was used in this study. Three types of bullets used were: Ruger® (ARX), Federal® (FMJ), and 2<sup>nd</sup> Amendment® (HP). Target materials were bicycle inner tubes, aluminum, and glass bottles. Three test fires were performed for each bullet type into each substrate at distances of five and ten feet. A total of 51 test fires were performed. The firing and firearm data were analyzed using the Analysis of Variance (ANOVA) and Student *t*-tests. Significance was determined when the  $p < 0.05$ .

The results showed that there was a significant difference in the size of the entrance hole when comparing a hollow point bullet fired into the three different substrates. There was a significant difference when using the different types of ammunition and comparing the bullet hole sizes in the various substrates. There was no significant differences when comparing the size of the bullet entrance and exit holes when these were obtained at the two different firing distances. Ruger® (ARX) bullet holes were more irregularly shaped than the circular-shaped bullet holes from the Federal® (FMJ) and 2<sup>nd</sup> Amendment® (HP). The shape of the 2<sup>nd</sup> Amendment® (HP) bullet holes in the rubber and aluminum were circular and symmetrical, while the bullet holes in glass were relatively rounded but irregularly shaped. In some of the target substrate, there were visual differences noted between the various bullet types used, but this was not investigated in the present study. In conclusion, with the increase in criminal use of firearms violence, it is especially important to obtain probative evidence and expert testimony from firearm examiners. It is recommended that using shorter muzzle-to-target distance be investigated in addition to the visual impact on the targets.

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#### Entry and Exit, Bullet Holes, ARX, FMJ, HP



## E48 Undergraduate Forensic Physics Education in Turkey

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**Learning Overview:** The goal of this presentation is to provide information about undergraduate forensic physics education in Turkey.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with: (1) a brief review of forensic science programs currently offered in Turkey; and (2) a detailed discussion of undergraduate physics education for forensic applications.

The Uskudar University Forensic Sciences Program (UUFS), established under the Faculty of Engineering and Natural Sciences, is the first college-level program in forensic science in Turkey. UUFS offers a natural sciences-oriented curriculum which mainly consists of introductory and advanced level mathematics, statistics, physics, biology, and chemistry courses. Half of the weekly course hours are allocated to laboratory trainings so that students are exposed to concepts, methods, and instrumentation that are commonly used in forensic science. There are also semester-long criminalistics, crime scene investigation, and law courses. The natural science courses are revised for forensic applications and taught as Forensic Mathematics, Forensic Physics, and so on. The program is currently the only available Forensic Science bachelor's program in Turkey. The curriculum satisfies Forensic Science Education Programs Accreditation Commission (FEPAC) standards.

Introductory forensic physics courses are divided into two semesters as Introduction to Forensics Physics I–II with accompanying laboratory sessions for students to gain hands-on experience in the field. The first part of the class focuses on the fundamentals of mechanics and provides theoretical background for forensic applications. Topics covered include: basic measurements, vectors, coordinate systems, Newton's Laws of Motion, conservation of energy and momentum, simple harmonic motion, simple and ballistic pendulum, and the fundamental applications of those topics. Students are equipped with a basic knowledge of physics and take a second course in physics that focuses on the physical basis of devices used during the investigation and evaluation of evidence. The covered topics in the Forensic Physics II class are fundamentals of optics, electromagnetic spectrum, nature of light, and alternate light sources and their applications in the field. UUFS does not consider the undergraduate forensic science degree as the terminal degree; hence more advanced forensic physics courses are provided in the graduate forensic science program.

The UUFS curriculum offers more than 20 elective courses, which enable students to specialize in various fields. These elective courses, such as Ballistic Examination, Shooting Incident Reconstruction, and Forensic Document Examination, helps students increase their knowledge in physics. The physics-based elective courses also have accompanying laboratory trainings; thus, courses include not only underlying physical principles but also direct and indirect applications of the underlying principles.

### Forensic Science, Crime Scene Investigation, Physics Education



## E49 Surgical Fire: A Case Report, Literature Analysis, and Medicolegal Considerations

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**Learning Overview:** After attending this presentation, attendees will understand the principles of a Surgical Fire (SF), its origin, its development, its consequences, and how to prevent it.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a key aspect of medicolegal implications of SF, analyzing each phase of the event before (propriety of informed consent), during (recommendations of best practices, protocols, and medical liability), and after (entity of lesions, necessity of reactive root cause analysis) the ignition.

SF is defined as fire that occurs in, on, or around a patient undergoing a medical or surgical procedure and is considered a “sentinel event.” Other sentinel events include wrong-site surgery and retained surgical items. These are “never events,” due to potentially serious or fatal consequences.<sup>1</sup> While data and reports published in Italy and Europe are lacking, in the United States, the prevalence of SFs has been estimated at approximately 600 events per year, and the total number of events is probably underestimated. The “fire triangle” theory is utilized for explaining the fire’s origin. Fire is composed essentially of three elements that are always present in the Operating Room (OR): (1) ignition—represented by electrosurgical units, lasers, defibrillators, etc.; (2) oxidizer—oxygen, nitrous oxide, medical compressed air, ambient air, etc.; and (3) fuel—alcohol skin preps, drapes/gowns, gauze/sponges, patient’s hair/skin, Endotracheal Tube (ETT)/nasal cannula, intestinal gases, etc. These devices and components are commonly used in the modern OR, and the complete elimination of fire risk is impossible. The American Scientific Society has provided recommendations of best practices to reduce the risk of SF.<sup>2</sup>

The reported case regards a 65-old woman with suspected thyroid carcinoma recovered in the University Hospital of Messina (Sicily, Italy). The patient was undergoing Video-Assisted Thyroidectomy (VAT) and suffered an SF during the wound closing surgical phase, which involved surgical drapes of the perioperative field. The surgical procedure was under general anesthesia (with ETT and 30% 2/L min O<sub>2</sub> supply). The fire was most likely produced by the ignition of the applied alcohol-based disinfectant (chlorhexidine gluconate) by monopolar electrocautery. Although the fire was promptly extinguished by the OR team, the patient reported disfiguring second- and third-degree burns to the antero-lateral surface of the neck.

An analysis of this case and a literature review suggest the following medical-legal considerations: informed consent must include the risk of this potentially high-grade surgical complication; and establish an Italian national best practice and intra-hospital protocol to calculate the fire risk score during surgery and develop a strategy for effective communication between the OR team (particular attention should be paid to the head/neck and pelvic regions, which are the most frequent regions that involve SF); finally, a reactive root cause analysis of each SF case should be done to collect data to identify health care improvement strategies. Current international best practices/strategies, designed to establish medical malpractice, include a delay of less than three minutes between application of an alcohol-based disinfectant and activation of electrocautery.

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### Surgical Fire, Burns, Medical Liability

## E50 Never Trust Appearances: A Case of Screwdriver Homicide

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**Learning Overview:** The goal of this presentation is to present an uncommon pattern of injury in a case of screwdriver homicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by focusing on the advantages of postmortem Computed Tomography (CT) and autopsy in studying uncommon patterns of injuries.

Lesions of the head caused by screwdrivers, while uncommon, are potentially lethal.<sup>1-4</sup> Skull bones are very resistant and most weapons usually break or bend when hitting the skull. Screwdrivers are much more resilient and are less likely to break or be deflected when striking the skull.<sup>5</sup> According to the literature, skull lesions caused by screwdrivers are most frequent in temporal (28.6%), parietal (23.8%), and orbital (9.5%) areas. The mortality rate of craniocerebral screwdriver injuries is approximately 47.6%. This presentation concerns a reported case of homicide committed by using a screwdriver, found embedded in the posterior part of the neck, simulating a fatal injury of the upper spinal cord. The forensic examination demonstrated that the cause of death had to be searched for elsewhere.

An 89-year-old man tried to commit suicide by jumping from the third floor of his apartment where he lived with his wife, who suffered from neuropsychiatric disorders. He was found still alive and transported to the local hospital, where he died a few hours later. The police entered the apartment where the man lived and discovered his 82-year-old wife lying on the bed with a blood pool under her body and a screwdriver embedded in the posterior part of the neck. The screwdriver was not removed to avoid potential neurologic damage, and she was transported to the hospital where she died a few minutes after her husband. At the apartment, a letter authored by the husband was discovered which begged forgiveness for his actions. No other weapons used on the woman were discovered in the apartment.

An autopsy was conducted on both bodies. The man's cause of death was confirmed as polytrauma due to a fall from height. In the woman's case, the autopsy was preceded by a total body CT, which revealed that the stem of screwdriver didn't hit the spine, but it was embedded in the soft tissue near the third cervical vertebra. Radiological examination revealed several fractures of the cranial bones and brain damage. The screwdriver stem protruded 5cm and, upon removal, was found to have a total length of 13cm. The layer-by-layer autopsy, which started from the neck, revealed diffuse hemorrhagic infiltration of the soft tissues, while the bony-ligament structures, cervical spine cord, and cervical organs were undamaged. There were 13 lacerations on the skull that were widely different from one another in shape and size. The bones on the right side of the skull buckled inward with several linear fractures in the parietal-temporal-occipital right bones. There was subdural hemorrhage of the left temporal lobe and bilateral subarachnoid hemorrhage of the parietal lobe and of the left parietal-temporal left lobe. The screwdriver produced a total of 13 lesions of different shapes and sizes. The radiological and the autoptic examination proved that the killing weapon was the screwdriver. The cause of death was not the penetrating injury of the neck as initially observed, but rather blunt force trauma to the head.

Penetrating injuries of the skull are most common in the thinnest bones, such as orbit and temporal. Screwdrivers are rarely used in assaults, but they can produce severe penetrating injuries. This case is peculiar because it is an uncommon case of homicide with a screwdriver found embedded in the posterior part of the neck. The screwdriver was indeed used to produce lethal blunt trauma to the head. Without discovering the screwdriver, identification of the single instrument used to inflict the injuries the multiple different lacerations of the scalp would have been very difficult. This case amplified a pitfall common in the forensic experience.

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### Screwdriver, Postmortem CT, Head Trauma



### E51 Accidental Autoerotic Death: An Unusual Case of Lethal Asphyxiophilia Associated With Autogynephilia

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**Learning Overview:** The goal of this presentation is to expose a case of lethal asphyxiophilia associated with multiple paraphilias, underlying the features that can be useful in a forensic investigation to exclude the possibility of sexual homicide or suicide in a case of suspicious autoerotic asphyxia.

**Impact on the Forensic Science Community:** Cases of autoerotic deaths are relatively rare in the forensic medical practice. In such cases, establishing the cause and manner of death is quite challenging for the forensic professionals. This presentation, due to the unusual methods used by the victim, will impact the forensic science community by providing important support for the management of similar forensic cases.

A 56-year-old man was found dead in the bedroom of his house, which was locked from inside, partially suspended over the edge of the bed by the neck and the torso while both the legs and the pelvis were lying on the mattress. The body was discovered with a diving mask over his head, dressed in female's clothing, including panties, bra, a corsage, and black stockings. Suspension was from a hook fixed to the wall behind the corpse, with a rope attached to a collar around the neck. The torso was suspended from the hook by two separate ligatures, one secured to a metallic wire that held two plastic funnels together fixed around the breast, and the other fastened to a leather belt placed at the waist. Pornographic pictures of bondage, depicting a set-up similar to the victim's, were displayed on the wall in front of the body. The deceased was divorced with one child, was employed, and had no diagnosed psychiatric illnesses. No suicide note was found at the crime scene.

During autopsy, two plastic discs were found under each funnel placed on the breasts. Both of the devices showed numerous pins with the sharp extremities pointed toward the skin. The male genitals were stretched by the means of a string knotted to another belt, fastened at the waist. There was marked facial congestion with massive petechial hemorrhage in the face and conjunctivae. The ligature mark was slightly oblique, pale, approximately cm wide, and encircled the neck above the thyroid cartilage, continuing up both sides of the neck to the occipital region where it ended. The internal examination showed hemorrhage in the area of the ligature mark that penetrated the thyrohyoid muscles. There were no hyoid bone or larynx cartilage fractures. All organs were congested, with petechiae on the epicranium, serosal surfaces of epicardium, and visceral pleura. There were no other significant findings at autopsy. Cause of death was mechanical asphyxia due to hanging while engaged in autoerotic activity.

Accidental autoerotic death is defined as a solitary, accidental death caused by a lethal paraphilia, such as asphyxiophilia. It is characterized by the use of a variety of techniques to produce the oxygen depletion needed to enhance sexual arousal, including hanging, self-strangulation, and suffocation with plastic bags or gas masks. This paraphilia could be associated with others, such as bondage, fetishism, and autogynephilia (male's propensity to be sexually aroused by the thought of himself as a female). In this case, the mechanism of asphyxia involved suffocation by a diving mask over the head and strangulation by neck ligature, combined with pain-stimulating agents, while wearing female's clothing. The peculiar variety and complexity of these mechanisms, combined together, made it difficult to exclude the possibility of a third-party involvement. However, suicide and homicide were excluded since all the characteristic features for an act of autoerotic asphyxia, described in the forensic medicine literature, were found thanks to a detailed crime scene investigation and the reconstruction of the victim's psychological history, which are of the uttermost importance in order to correctly establish the nature of an autoerotic death.

#### **Autoerotic Death, Asphyxiophilia, Autogynephilia**



## E52 School Bullying Affecting America's School Children: A Look at Statistical Trends

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**Learning Overview:** After attending this presentation, attendees will understand bullying by school-aged children and the perceived psychological effects it has on victims, the perpetrator, and the community. The goal is to provide a statistical insight on bullies, their home life, and their effects.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering insight into current statistical data collected from the 2018–19 and 2019–20 academic years for primary-level school administration and historical timelines over the past five years to showcase a trend in bullying and its effects.

Bullying in every form has continually received growing attention as the serious nature of violence perpetuates into extreme forms. Bullying has become more reliably recognized as empirical research is being conducted. This research has fixed its attention on the underlying characteristics and behaviors of both the school bully and their victim(s), and how professional school administrations recognize these traits. Two questions that attendees will be presented with are: (1) Are there any programs being utilized to counteract the ongoing problem of bullying or to prevent it from happening?; and (2) Is there a trend that can be identified with bullying and is it possible for administration to step in without violating civil rights?

With more than 250 people dying in active shooter and mass casualty events since the Columbine shooting, there is a need to place these characteristics and behaviors into the framework of the situation in which they occur to expand the research and allow for the expatriation of more in-depth and inclusive educational, prevention, and intervention programs.<sup>1</sup> This research focuses on professional school administrators in southwestern Oklahoma to identify the signs of bullying and determine if current school bullying education, prevention, and intervention is successfully administered. If not, can there be a change implemented and what change should occur? A look into what programs should be available and funding possibilities to help assist the schools will also be explored.

The goal of this research and presentation is to provide forensic psychological insight to the warning signs and predispositions of bullies. This includes their home life, social habits, and school life to better understand what drives a bully to bully. Understanding motivation is essential since most past research concentrated on the bully after he/she commits the behavior instead of what causes them to act in such a manner.<sup>2</sup> In turn, this research should show a consistent statistical data trend to help identify triggers and warning signs that can be changed before the incident(s) occur and develop into a deadly scenario without violating civil rights and privacy to all individuals involved.

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### Active Shooter, Forensic Psychology, Bullying





### E53 Choose the Cause of Death: A Complex Suicide

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**Learning Overview:** The goal of this presentation is to present a multidisciplinary approach to a case of complex suicide. In order to assist the judicial authorities and police inquiries in classifying the crime, toxicologists and forensic pathologists have to be involved. In this case, the role of the forensic pathologist was of primary importance to clarify time and causes of death and to distinguish between suicide and murder.

**Impact on the Forensic Science Community:** Complex suicides are an uncommon form of suicide in which multiple suicide methods are associated, representing a challenge for forensic pathologists. They are classified as typical (two suicide methods associated) and atypical (more than two methods involved) forms of suicide. In the case of a complex crime scene like this, the differentiation between homicide and suicide can be difficult; this presentation will impact the forensic science community by providing important support for the management of similar forensic cases.

This case report is about an atypical, complex case of suicide. This 55-year-old woman was found by her sister, deceased in her bedroom. The sister immediately called for medical assistance, police, and, ultimately, the medical examiner was notified.

The woman was discovered lying on her bed with her left hand tied to her right foot with Scotch® tape. Three plastic bags were wrapped around the head. She had superficial incisions on her left wrist. There were bloodstains on the floor, which led to the hypothesis that the victim moved after suffering injuries. A bedside table was leaning against the door, inside the room. There were many empty packages of drugs (acetaminophen, levothyroxine, diazepam, and Acetylsalicylic Acid [ASA]) found in the bedroom.

A multidisciplinary forensic approach, including full autopsy, histological, immunohistochemical, and toxicological analyses, were performed. The autopsy revealed pulmonary and cerebral edema. In addition, thrombosis of the left middle cerebral artery was observed, occluding 80% of the vascular lumen. Histological analysis confirmed the autopsical evidences of pulmonary and cerebral edema and showed massive acute emphysema. Histological analysis of the heart revealed severe myocarditis and advanced coronary disease. Brain tissue showed signs of subacute ischemic suffering. The liver showed signs of centrilobular necrosis. Toxicological analysis revealed a lethal concentration of acetaminophen (4,026.2µg/ml), and therapeutic concentrations of diazepam. The results of autopsy and subsequent examination, combined with the result of crime scene examination, determined the cause of death as smothering. There were not signs of forced entry and no defensive wounds. The absence of these elements eliminated homicide as a manner of death. These findings suggest that a methodological approach, which involves forensic pathologists, toxicologists, and crime scene investigation is essential in cases like this. This collaboration properly identifies the cause and manner of death.

#### Complex Suicide, Toxicology, Asphixiology



## E54 Strategies for Reconciliation of Personal Identifying Information and DNA Profile Data at a State DNA Index System (SDIS) Databasing Laboratory

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**Learning Overview:** After attending this presentation, attendees will be aware of how the Texas State Combined DNA Index System (CODIS) Offender Databasing Laboratory addresses unique challenges with known offender samples to include: (1) sex/gender discrepancies between reported biographical data and DNA profile data; (2) twins in the Texas State CODIS Offender Database; and (3) multiallelic offenders and/or chimeric DNA profiles.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the complexities of known offenders' biographical data and DNA profile data and how these complexities can affect casework and/or law enforcement interpretations.

The Texas Department of Public Safety (DPS) CODIS Offender DNA Databasing Laboratory (Texas SDIS Lab) encounters challenges that differ from traditional forensic DNA casework laboratories. Because the main role of CODIS is to provide law enforcement with investigative leads, accurate identifying information is emphasized. Biographical data provided with each sample is compared with crime record data to verify identification prior to processing their DNA sample. In some cases, the information obtained from the DNA profile leads to questions about the offender's reported biographical data. The following discrepancies are rare but important to investigate for internal laboratory quality assurance: accurate familial searching and providing accurate identifying information associated with a CODIS hit.

Sex/gender discrepancies are queried in the internal Laboratory Information Management System (STACS-DB from STACS DNA, Inc.) to yield discrepancies between biological sex data collected during DNA analysis and biological sex data manually entered into the database during sample data entry. Data entry errors were resolved without retesting. Retested samples included 81 Fast Technology for Analysis (FTA) blood samples, 67 non-FTA blood samples, 40 buccal samples, and nine samples on GenTegra® GenPlates®. All samples were retested using GlobalFiler® Express amplification kit and a portion tested for Y-chromosomal Short Tandem Repeat (Y-STR) using the Y Filer® kit. Following processing, amelogenin, DYS391, and Y-chromosome insertion/deletion (Y-indel) loci results for each sample were compared with the reported biological sex. While the project is ongoing, results suggest that there are sex-typing issues as a result of genetic insertions, deletions, and primer site polymorphisms at amelogenin, or other genetic anomalies, such as XXY females. Of the portion tested for Y-STR, 12% of male samples exhibiting apparent female STR profiles produced a Y-STR profile consisting of 12, 14, and 15 null loci. Results also suggest that reported sex may contradict with DNA sex based on an individual's self-identification. Many discrepancies were in fact updates to reported sex in crime records as an individual changes the way they report their identity at arrest, such as in a case of gender dysphoria.

Texas currently has over 950,000 offender and arrestee samples in SDIS and receives approximately 3–7 sets of identical twins each month. Possible sets of twins are initially identified when two individuals in Texas SDIS appear to have identical DNA profiles. First, the samples are researched to rule out duplicate samples that were accidentally processed as two separate individuals due to biographical data errors within crime records. The possibility of twins is then researched through contacting supervising agencies and sporadically analyzing requested vital records. As of 2018, over 800 sets of identical twins exist in Texas SDIS. Currently, there is no method within CODIS to differentiate individuals from a set of identical twins. When a match occurs with twins, both individuals are reported to the investigating agency and the local lab working their forensic case.

Chimeric offender samples are rare in Texas SDIS, with just 22 chimeric samples identified as of 2018. However, a chimeric profile could mislead an investigation if the sample is interpreted as a mixture and devolution is performed, when in fact the source of the DNA is from one contributor. Explanations vary from embryonic development to bone marrow transplants. Chimeric or mixed profile offender samples are initially identified at analysis. Once contamination is ruled out, an additional DNA sample is requested from a supervising agency, if possible. If the original sample was a blood sample, a buccal sample is requested to compare DNA derived from different biological material. Once confirmation testing of the mixed profile is performed with a second collection, the sample is uploaded into CODIS to be searched in the Multi-Allelic Offender (MAO) specimen category. Information about the research into these samples is documented internally.

### Databasing, Gender, Chimeras



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## E55 Sex and Race Determination Based on Attenuated Total Reflection/Fourier Transform Infrared (ATR/FTIR) Spectroscopy of a Bloodstain

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**Learning Overview:** After attending this presentation, attendees will gain knowledge about: (1) the limitations of current methods for bloodstain analysis during forensic investigation; (2) the importance of human phenotype profiling for forensic purposes; (3) the significance of a non-destructive method for examination of trace evidence at a crime scene; and (4) the advantages of FTIR spectroscopy in forensic investigation. The goal of this presentation is to disseminate results of FTIR spectroscopy for bloodstain examination as well as the results of chemometrics for distinguishing between human sexes and races.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by disseminating results of the non-destructive and rapid human sex and race determination from a bloodstain at a crime scene immediately after discovering a crime scene.

Human phenotype profiling is one of the most important analyses during a forensic investigation. It can play a crucial role in the early stages of investigation. All current methods of phenotype profiling based on the analysis of body fluid traces are destructive to the sample and, most importantly, need to be performed in a lab and not at a crime scene.

In this study, ATR/FTIR spectroscopy was evaluated as a potentially non-destructive, rapid, and *in situ* method for discriminating sex and race based on the analysis of human bloodstains. It is known from the literature that the biochemical composition of blood changes with donor sex and race. ATR/FTIR spectra were acquired from dry bloodstains, and an advanced statistics methodology was utilized to enhance the differentiation capability of the method. Specifically, Partial Least Squares Discriminant Analysis (PLSDA) was employed to create models for differentiating Caucasian (CA), African American (AA), and Hispanic (HI) donors according to their sex and race. This approach was evaluated by subject-wise Leave-One-Out Cross-Validation (LOOCV), resulting in a greater than 90% correct classification. In addition, the main models were validated externally with four hold-out samples, which were not used for the training data set. The validation with hold-out samples resulted in 100% accuracy for both sex and race predictions at the donor level.

Overall, this proof-of-concept study demonstrated the great potential of ATR/FTIR spectroscopy and chemometrics for phenotype profiling for forensic purposes based on dry bloodstains. Translating this technology to a portable instrument would provide an excellent opportunity for conducting human phenotype profiling immediately after the crime scene is discovered.

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### Bloodstains, Phenotype Profiling, Nondestructive Analysis

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**E56 A Chemical and Biological Analysis of a Medieval Skeletal Collection From the Archaeological Site at Lohor, Croatia**

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**Learning Overview:** After attending this presentation, attendees will have an overview of the importance of forensic analysis methods application on ancient skeletal remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by giving insight into the challenges and cooperation of both archaeology and forensic sciences because it deepens interdisciplinary communication and collaboration, taking a step forward in the reconstruction and the interpretation of such results.

Material culture is the primary source of information in archaeological science. The possibility of improvisation and understanding of a holistic approach generates a range of new connections between both areas. The true direction is what must be seen as the final goal of the study of materials in archaeology using forensic methods. During archaeological research in Lohor in 2011, three different sections of cemetery burials were examined. The first was to the east, outside the enclosure wall of the shrine of Our Lady of the Mountain. The middle and most intriguing burial phase included individual graves of five persons. This group of skeletons from the 11<sup>th</sup> century were discovered in an unattractive part of the cemetery, positioned behind the front end or on the North side of the pre-Romanesque church. The skeletons were found in a contracted and a semi-contracted position.

The primary anthropological identification, which includes sex, age at death, and stature determination, together with the analysis of bone pathological conditions and the development of musculoskeletal attachments have provided an abundance of data. The anthropological analysis determined all five skeletons were male and ranged in age from juveniles to a nearly senile phase. Stature varied from medium to tall, medium to robust, with a wide shoulder breadth. Pathological changes on bones diagnosed as leprosy were visible on all skeletons, with only one skeleton highly impacted.

Multiple stable isotope analysis (<sup>13</sup>C, <sup>15</sup>N, <sup>18</sup>O) has provided an insight into the personal migrations and the chemical description of the regions where these persons spent their early childhoods, as well as of the regions that marked the last years of their lives. Chemical isotope inspection of individuals revealed that the M<sub>1</sub> tooth was enriched in comparison to the bone in <sup>13</sup>C and <sup>18</sup>O isotopes but not in <sup>15</sup>N isotopes. The lack of an enrichment of nitrogen leads to the conclusion that the mother's diet was likely poor in animal proteins. The carbon values are higher in one sample than others, indicating a higher diet of C<sub>4</sub> plants. In contrast, other individuals displayed enrichment in the M<sub>1</sub> tooth in <sup>18</sup>O and <sup>15</sup>N and depletion of <sup>13</sup>C. The mother's diet was in part based on C<sub>4</sub> plants, while that of the adult was based only on C<sub>3</sub> plants.

The values calculated for the ingested water are rather negative, indicating a provenance from continental and mountainous inland areas. The skeletal and dental material from each grave was sampled for ancient DNA analysis, which was performed with the Investigator<sup>®</sup> 24plex QS Kit for human identification to gain information of possible kinship within a family group. In total, five male DNA profiles were analyzed that were not related to one another. For further kinship statistical analysis, Y-chromosome or mitochondrial sequence analysis should be considered on a larger number of skeletal remains.

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**Stable Isotope Analysis, Anthropology Analysis, DNA Analysis**

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**E57 Evaluating Medicolegal Examinations of Turkish Detainees During the Recent State of Emergency in Turkey Within the Scope of the Istanbul Protocol**

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the recent human rights violations in Turkey and the importance of the Istanbul Protocol for detecting torture.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by drawing attention to the importance of the Istanbul Protocol for protecting human rights in the recent State of Emergency.

Maltreatment and torture during detention continues to be a serious problem in many countries. For this reason, medical evaluations of maltreatment and torture should be performed appropriately. The Istanbul Protocol is a United Nations document guiding doctors and legal experts in terms of investigating and reporting torture. The protocol provides minimum standards for investigating torture claims, examining potential torture victims, and documenting physical and psychological evidence for torture. After a failed coup d'état in Turkey on July 15, 2016, a state of emergency was declared; 150,348 people were dismissed from their professions, while 500,650 people were detained. To date, 30,947 people are still jailed. This present study will evaluate the reported medical examination procedures in Turkish detention facilities of Turkish detainees finding asylum in Germany after their detention in Turkey, the present process of medicolegal reporting, will evaluate the compliance to the Istanbul Protocol, and discuss the issue in the light of literature.

Adult Turkish citizens who sought asylum in Germany after the 2016 coup attempt in Turkey were eligible to participate in this study. Participants were recruited via announcement in a mobile messaging support group for Turkish citizens in Germany. The study participants were asked questions regarding the examination steps stated in the Istanbul Protocol. The questionnaires were created with Google® Forms and were sent to the participants via mobile messaging applications. Descriptive statistics were used to describe the data.

Fifty-one people participated in the study. Forty-five (88.2%) participants were male, while 6 (11.8%) participants were female. Considering the educational background of the participants, 29 (56.9%) participants had a bachelor's degree, 13 (25.5%) had a master's degree, and 9 (17.6%) had a PhD. Most participants were in the age range of 36–45 ( $n=24$ , 47.1%), while 19 participants were under the age of 36. Twenty-five (49%) participants were not asked for their personal information before examination. Forty-five (88.2%) participants stated that police officers were present during medical examinations. The examinations lasted between ten seconds and ten minutes and 29 participants (56.8%) were examined in less than five minutes. The statements of 36 (70.6%) cases related to the detainment were not requested. A detailed examination regarding maltreatment or torture has not been performed on any participant. Considering the location of the medical examination, 6 participants (11.8%) were examined in a hall, 1 (2%) was examined in a police car, and 24 (47%) were examined in jail or a police station. While 7 participants (13.7%) stated that they had not been exposed to any kind of physical maltreatment, the other 42 participants (82.3%) had been exposed to maltreatment, such as beating, handcuffing, extended periods of standing, sleeping on bare concrete floors, imprisonment in confined spaces without fresh air, or psychological torture and maltreatment.

The practice of torture and maltreatment seems to continue despite all international attempts of prevention. When one considers the fact that thousands of people were detained for similar reasons during the recent state of emergency in Turkey, the impact of this event is evident. The most significant points to prevent torture are the medical examinations made during detention. The responses given by the participants revealed that not all medical examinations during detention were performed in accordance with the Istanbul Protocol. For this reason, it is believed that proper physical examinations would have been legally problematic for involved officials. In this respect, practicing the appropriate medicolegal examination in accordance with the Istanbul Protocol and sharing those reports are of great significance. Because this study only included a small number of participants, further studies are needed to draw definite conclusions and to avoid sampling bias.

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**Istanbul Protocol, Torture, Detention**

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## E58 The Medicolegal Evaluation of Detention Procedures During the Recent State of Emergency in Turkey

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**Learning Overview:** After attending this presentation, attendees will better understand the recent human rights violations in custody in Turkey through the eyes of a well-educated group of people who migrated to Germany from Turkey.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by drawing attention to troubles faced by well-educated refugees who migrated to Germany from Turkey.

The United Nations defined torture as infliction of physical or psychological harm by public officials or other persons acting in an official capacity. According to 2013 data from the United Nations, more than 50 million people are affected by forced displacement as a result of conditions such as war, oppressive regimes, or natural disasters. The rate of exposure to torture among those people is estimated to be between 5% and 35%. It is predicted that there are between 3–17 million victims of torture among people affected by forced displacement. However, a large portion of torture victims continue to live in their home counties where they are exposed to torture. In Turkey, the number of people directly exposed to torture is predicted to be several million. During the recent state of emergency in Turkey, many academicians, journalists, teachers, and doctors left Turkey as refugees, and mostly chose Europe to apply for asylum because of geographical closeness and a large Turkish community. This present study discusses the medicolegal examination of detainees in Turkey.

Eligible participants consisted of Turkish citizens aged 18 and older who fled Turkey during the recent state of emergency and applied for asylum in Germany. The participants were recruited with an announcement in a mobile messaging group which functions as support group for Turkish citizens in Germany. The participants answered a questionnaire, including demographical data and detention procedures, without disclosing their identity. Google® Forms was used to conduct the questionnaire with the participants.

A total of 294 people participated in the survey. Males comprised 284 (84.4%) of the participants, while 46 (15.6%) were female. There were 91 (31%) teachers, 30 (10.2%) academicians, 10 (3.4%) doctors, 33 (11.2%) members of the army or police, 19 (6.4%) businessman, 16 (5.4%) lawyers or prosecutors/judges, and 23 (7.8%) were from other occupational groups. All participants stated that they had not been previously sentenced or detained. When the educational backgrounds of the participants are considered, 166 (56.5%) participants had a bachelor's degree, 66 (22.4%) had a Master's degree, and 40 (13.6%) had a PhD or specialty in medicine. Researchers found that 91 (31%) participants were detained for four to seven days, while 100 (34.0%) participants were detained longer than seven days. Lawyers were not available for 120 (40.8%) of the participants and 257 (87.4%) were unable to meet with family members.

Physical trauma was reported by 57 (19.3%) participants during detention, while 219 (74.5%) participants stated that they were subjected to psychological violence. Most participants stated that they were refused medical help ( $n=273$ , 92.8%). Adequate nutrition during the detention process was not provided to 248 (84.4%) participants, while 155 (52.7%) participants pointed out that they were exposed to water restrictions. Restricted washing facilities were reported by 219 (74.4%) participants, while 166 (56.5%) participants reported inadequate access to toilets. Other reported measures included constant lighting ( $n=182$ , 61.9%), unsuitable cooling or heating ( $n=168$ , 57.2%), exposure to loud music ( $n=28$ , 9.5%), or verbal abuse ( $n=161$ , 54.8%).

In this present study, the reported detention procedures and practices do not comply with human dignity and appear to be a violation of the United Nations convention against torture. This cross-sectional study indicates that further studies regarding those practices should be conducted and these processes should be illustrated in detail. Because the study only included a small number of participants, further studies are needed to draw definite conclusions and to avoid sampling bias.

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### Turkey, Detention Procedures, Torture



### **E59 Health Care Professionals as Persons of Interest? Preventing Strategies, Medical Liability, and Italian Jurisprudence Through the Analysis of an Inpatient Psychiatric Suicide**

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**Learning Overview:** The goal of this presentation is to analyze preventing strategies in cases of inpatient suicide through a case report and a review of international and Italian jurisprudence, to reflect about risk management and prevention, but also to highlight the critical elements related to this highly debated theme.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting an overview of the medicolegal aspects of inpatient suicide, focusing on medical liability and risk management.

Sentinel events are unexpected occurrences involving death or serious physical or psychological injury, or the risk thereof. In-patient suicide is a sentinel event that can be significantly reduced by adopting specific prevention measures. Literature recommends accurately registering medical history at admission to stratify the risk of suicide and level of surveillance required. Published authors have stated the most important prevention strategy is related to eliminating the physical instruments that could be potentially used for self-harming.

This case report concerns a 43-year-old woman suffering from severe anorexia who had been treated with an antipsychotic and who recovered. At admission, her Body Mass Index (BMI) was 12.3Kg/m<sup>2</sup>, compared with a normal BMI of 20–25 Kg/m<sup>2</sup>. She was aware of her severe physical condition, which led to her acceptance of the therapies required for treatment. She was initially fed by parenteral nutrition, but developed anxiety and suicidal thoughts related to this treatment; as a result, antipsychotic therapy was initiated. She also had daily interviews, but she refused to take part in group therapy. After initial improvement, her psychical condition worsened. She tried to injure herself with an insulin needle and refused to eat. After these episodes, surveillance was initiated during meals, and her room was thoroughly searched. Medical staff found scissors, a lancet, and a telephone wire. The telephone wire had likely been passed through the window despite the window handle having been removed. Recommendations included observing her visitors and continuation of antipsychotic therapy.

One night, approximately six months and two weeks after admission, the patient was reported to have used the toilet several times. About 6:00 a.m., nursing staff could not locate her. Local police were notified about 7:00 a.m. About 4:55 a.m., a human body was discovered on the tracks of the local train station. An underweight female, wearing pajamas, a nightgown, a pair of slippers, and a cannula was observed lying prone with her head on the track and the remainder of her body next to the track. The woman had cranial burst fracture with cranial vault avulsion and brain exposure.

The woman was quickly identified as the missing psychiatric ward patient. An examination of her room revealed that the window of her room had been opened, using the handle that had been previously removed and presumably hidden. An external examination at autopsy confirmed the injuries were consistent with a train accident. The time required to travel to the train tracks, the victim's medical records, and other circumstantial data suggested a gap of approximately five hours in patient surveillance.

Health care liability in cases of inpatient suicide arises from the duty to supervise patients who are under care. Prevention can significantly reduce the number of inpatient suicides, but some strategies are not always viable because of limited resources or are inefficient due to the unpredictability of suicide. The risk of self harm is not always foreseeable. The Italian criminalistic jurisprudence concerning in-patient suicide is unclear. Health care personnel are sometimes cleared of wrongdoing in consideration of the right of freedom and self determination by the patient; however, it most frequently recognizes medical liability due to the duty of the medical staff to protect and look after patients. This case has not been sentenced yet, but there is high probability that health care professionals will be charged for the death of the patient due to the inadequate surveillance and the organizational gaps.

#### **Medical Liability, Inpatient Suicide, Prevention**

## E60 A New Approach and Suggestions for Child Sexual Abuse Cases

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**Learning Overview:** After attending this presentation, attendees will understand a new approach in child sexual abuse and examination as well as reporting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of children's rights, sexual abuse cases, and all dimensions of the forensic aspects.

The World Health Organization (WHO) defines child sexual abuse as “the involvement of a child in sexual activity that he or she does not fully comprehend and is unable to give informed consent to, or for which the child is not developmentally prepared, or else that violate the laws or social taboos of society.”<sup>1</sup> Children are exposed to abuse in many countries. Reports of child sexual abuse cases have risen considerably in recent years.<sup>2</sup> Child sexual abuse is usually associated with other violence, such as physical violence, threats, frightening, drugging, or “obtaining consent” from individuals who are unable to do so, either because of age or mental disease.

The management of cases of sexual violence is a complex, long, and difficult process from the first encounter to the completion of treatment and rehabilitation of the patient. The process requires a multidisciplinary approach, with emphasis on the health and well-being of the patient. The process of disclosure is very difficult for children.<sup>2,3</sup> The investigation begins after being notified of an allegation of child sexual abuse. Some investigative practices lead to the identification of other victims or to allegations of False reports. Because of these factors, the forensic investigation must consider the need of the child and the accused person.<sup>4</sup>

During an 18-month period between 2018 and 2019, 156 sexually abused children, all below the age of 15 years, were examined and evaluated at the Cukurova University Forensic Medicine Department. Turkish Medical Clinics categorizes children below the age of 15 years as pediatric patients. Similarly, under Turkish law, children below the age of 15 years are unable to consent to sexual activity. The cases examined included 111 (71.2%) females and 45 (28.8%) males. The children's ages ranged from 4 to 15 years, with the average age of 11.78 years. There were 79 (50.6%) cases of repeated abuse and 77 (49.3%) cases of one-time abuse. There were 45 (28.8%) cases of reported vaginal abuse, 45 (28.8%) cases of anal abuse, and 66 (42.3%) cases of sexual touching. Most of the victims were female, and 69.8% of the time they recognized the male perpetrator. Mental retardation was present in 7% of the victims. Examinations of victims took place in the first 72 hours in 41 (26.3%) cases of abuse. Of those examinations, 39 (25%) had positive physical findings during clinical examination. All examinations were completed both with traditional visual clinical forensic sexual victim examination and by digital multispectral video colposcopy to evaluate and document the case properly for court. The type and frequency of the abuse was obtained from either the victim's history, the health care facility, or some other official entity.

Child sexual abuse usually impacts the victim during childhood, adolescence, and even adulthood. The long-term impact can compromise the life of productivity as a result of the maltreatment of the victims. Sexual assault victims should not be repeatedly traumatized by clinicians and other official authorities. For this reason, a new clinical examination technique and psychiatric evaluation methodology has been implemented. The major point should be the victim's higher benefit, and this should always be protected.<sup>5,6</sup> A well-trained clinical forensic examiner should always complete proper methodologies at a proper medical facility, and other professionals should always consult with clinicians.<sup>2,4,7</sup> The rights of children and women should always be accepted as a part of human rights. Society working as a whole should understand the unity of the body of a child or woman.<sup>2,5</sup>

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### Children Rights, Sexual Abuse, Clinical Examination



## E61 An Evaluation of Child Suicide Death Cases

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of child deaths from suicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness and identifying children at risk for suicidal behavior.

Adolescent suicidal attempts are a serious public health concern. It causes psychiatric, economic, and physical problems. The incidence of suicides increases rapidly until the age of 18 and accounts for 1.5% of all deaths worldwide, making it the tenth-leading cause of death.<sup>1</sup> It is the third-leading cause of death among children and adolescents aged 10 to 18 years.<sup>2</sup> The most prevalent factors of adolescent suicide are family conflict, school-related problems, bullying, impulsivity, and depression.<sup>3</sup> Psychiatric disorders in young adults significantly increase the risk of suicide. These individuals are more affected by relationship problems. Although suicidal thoughts and suicidal behavior are rare before puberty, there is increasing concern about identifying individuals at risk for suicidal behavior.<sup>2</sup> This study examined the socio-demographic characteristics and forensic medical significance of suicidal child death cases.

This study examined 204 suicides from 2018 that were autopsied at Adana Group Presidency of Forensic Medicine Institution. This study evaluated age; gender; suicide method; suicidal environment; dates; presence or absence of a diagnosed psychiatric disease; and the presence or absence of prior suicide attempts. The review consisted of the postmortem examination and autopsy records.

The youngest age at suicide was 12, and 20 cases (9.8%) were under age 18. Males comprised 75% ( $n=15$ ) and females comprised 25% ( $n=5$ ). Suicide occurred in domestic settings in 80% of the suicides. The most common month was September, with 20% of the suicides occurring that month. Thursday was the most common day of the week with five cases (25%). Three cases (15%) had received prior psychiatric treatment. Two cases (10%) had previously attempted suicide. Hanging was the most common method of suicide (35%), followed by jumping from height (30%), firearms (20%), and self-poisoning (15%). Firearms were the second preferred method of suicide in adults, while children chose jumping from height as the second most common method. This study hypothesizes that the inability of children to obtain firearms led to the choice to jump.

There are effective techniques for lowering suicide rates.<sup>4</sup> Once someone attempts suicide, the risk of attempting suicide again may increase. Society and the State must adapt their approaches to suicide. The most important thing to do in this regard is to restrict access to the means of suicide (firearms, chemicals, etc.).<sup>1</sup> The media should avoid suicide contagion by avoiding front-page coverage and sensationalizing suicide, and can assist by providing information on treatment resources. Suicide screening at schools can identify adolescents who have mental health problems. The accurate diagnosis of psychiatric disorders and their successful treatment can significantly reduce suicide rates. Treatment of psychiatric patients should be financially and morally supported; health professionals should be trained on the subject; maximum sensitivity should be shown during the rehabilitation process; and patients should be returned to the community as healthy individuals.<sup>5,6</sup> Literature highlights the noticeable existence of childhood suicide, although oftentimes unstudied. Recognizing psychic distress and suicidal behavior in childhood is extremely necessary for children to have adequate psychiatric treatment.<sup>7</sup> Protocols should be developed for the early recognition of suicidal behavior. Health professionals and teachers should be empowered to be able to help children with suicide risk predisposing signs.<sup>6,7</sup>

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### Child Death, Child Suicide, Suicide Prevention



### E62 Rapid, Real-Time, and In-Field Detection of Fentanyl Residue: A New Approach Using Ion Mobility Spectrometry (IMS)

John Z. Wang, PhD\*, Artesia, CA 90701

**Learning Overview:** After attending this presentation, attendees will understand a new technology to detect fentanyl residue (<10ng) in a rapid (<5 seconds), real-time (instant peak display), and in-field (a portable device) manner.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a cutting-edge method of detecting opioid-fentanyl-based drugs in the field. Attendees will learn how the IMS is capable of displaying the detection results in less than five seconds and also related testing procedures, which may have a strong implication for law enforcement and public health agencies in addressing the current fentanyl crisis worldwide.

Currently, it is argued that opioid-fentanyl is the number one enemy in the opioid crisis and costs thousands of lives each year in the United States. Due to fentanyl's potent nature, law enforcement and public health agencies need a portable technology to determine its existence in certain situations, such as crime scenes, dead bodies, mail parcels, and/or abandoned drug apparatus. A new portable technology is based on the principle of IMS, weighs only 5kg, and can provide a real-time detection result on fentanyl residue in less than five seconds in field tests. Further, the technology can be connected to a Wi-Fi system for a remote communication. Finally, the IMS system has nine unique functions similar to the capacities of a Mass Spectrometry(MS) system.

With a quasi-experimental study by black box sampling, two spots on a school backpack were located for testing purposes: one spot (1mm<sup>2</sup>) was dipped with a drop of fentanyl (medical use) by a syringe, and the other spot (1mm<sup>2</sup>) with a drop of water. Two glean wipes on both spots using two separate swabs (a special material) were inserted into the machine inlet slot. The device was able to provide both readings within five seconds with accurate results. The system's internal cleaning process between two tests takes about 30 seconds. Therefore, the two consecutive tests require just under one minute on an area of less than 1mm<sup>2</sup> for less than ten Nano-grams of fentanyl in concentration under a normal atmosphere condition.

This IMS system consists of two parts: (1) the compounds that are used in testing must be ionized to become ions; (2) the ions pass through an electrical field so that different types of ions will be separated by the size, charge, and mass of the compounds. It is similar to the MS method except that IMS is a portable system and does not require a vacuum. The detection happens in a normal atmospheric environment so that it is relatively less expensive and easily made as a portable detector in the field. In fact, its detection sensitivity may reach the one Nano-gram level, depending on the types of fentanyl tested, which is quite adequate for detecting fentanyl variations on international and domestic parcels in real-life postal inspections. One of the special features of this technology is its use of Very Deep Ultra-Violet (VDUV) photons to ionize the sample, instead of a traditional radioactive ionization source, such as Ni63 isotope material. Therefore, it is non-radioactive and thus very safe to operate in the field. Finally, the drift time  $t$  can also be deducted into a  $kO$  parameter, which is the universal constant for each individual compound ( $kO$  is a physical parameter determined by the physical nature of the molecule/ion).

This study hypothesizes that a rapid, real-time, and in-field determination of fentanyl is one of the technical challenges in fighting the current fentanyl crisis in the United States. From this study, it is concluded that the application of the IMS is able to provide a practical method at the scene, in the lab, or even during courtroom testimony. This study strongly contends that the study results add a new effective approach for law enforcement and public health agencies. It is believed that if such technology can be utilized as a common practice, our duties and performance in addressing the fentanyl crisis will certainly be advanced from the past toward a more science-based future.

**Fentanyl Detection, Ion Mobility Spectrometry (IMS), Forensic Toxicology/Chemistry**



## E63 Forensic Palynology: Pollen and Its Role in Crime Scene Investigation, National Security, and Forensic Science

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**Learning Overview:** After attending this presentation, attendees will be familiar with the area of forensic palynology and how pollen and spores have been recently used within the forensic science community to assist with determining postmortem intervals, investigating major crimes, and assisting with national security issues.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing awareness to a subfield of forensic science that is up and coming within the United States. By providing awareness to this field, the forensic science community will be able to see how pollen has been used to solve crimes around the world and the benefits it can bring by being utilized in forensic casework and on-going investigations.

Forensic palynology is an up and coming discipline within the forensic science community. A science that has been utilized in New Zealand is now being used across the globe in Australia, the United Kingdom, and the United States. Forensic palynology uses spores, pollen, and other acid-resistant microscopic plant bodies to assist with criminal and civil cases.<sup>1</sup> The results from the analysis are used to help aid investigators by providing information that links to certain geographic locations. Forensic palynology has also been used in cold case investigations, postmortem interval estimations, and national security issues. Though normally used for outdoor scenes, more research is being conducted on the value of pollen analysis in crimes such as forgery and fraud. Although this science is not prevalent in the United States, it is slowly becoming more recognized through its recent use in cold case work and its current use with national security and organized crime cases with the United States Customs and Border Protection agency.

One of the most recent cases in which forensic palynology was used in the United States was the Bella Bond case or, at the time, the Baby Doe case. An unidentified female was found in the Boston Harbor area of Massachusetts. By utilizing forensic palynology, scientists were able to trace the pollen found with the body to the northeast region, specifically around the Arnold Arboretum in Boston, MA.<sup>2</sup> This data assisted investigators by providing a geographic location of where the child lived, or was, prior to her death.

Pollen has the ability to withstand most acids and the decay process, which allows it to be found on skeletal remains or heavily decomposed bodies.<sup>1</sup> Due to pollen having these properties, studies have been conducted to try to see if pollen analysis can be used to determine a postmortem interval with skeletal remains, specifically trying to determine a potential season of death. One of the first studies conducted with pollen analysis was on skeletal remains to try to determine a postmortem interval in Magdeburg, Germany, in 1994.

Using pollen analysis, investigators are able to determine where illegal substances are being grown or produced and are able to test the substances to see if they are being locally grown or imported. In one case study, investigators were able to determine that the substance was locally grown and they were not dealing with a larger drug trafficking issue.<sup>3</sup> The analysis of marijuana and trying to link the origin of it became very successful, which has then led to pollen analysis being used in other cases involving illegal substances, such as cocaine and methamphetamines.<sup>2</sup>

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### Forensic Investigation, Pollen, National Security



## E64 A Forensic Comparison of Cable Ties to Create a Database

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**Learning Overview:** The goal of this presentation is to show the variation in characteristics with cable ties and their potential usefulness as physical evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the foundation for a cable tie database. This database could eventually provide valuable investigative leads in forensic cases.

Cable ties are often overlooked as forensic evidence since their characterization has not been extensively studied. However, cable ties may be used in criminal events in a variety of capacities, such as binding the hands of victims, in the design of homemade explosives, and during strangulations. Cable ties are made by injecting molten nylon plastic into metal molds. Two metal plates with a certain number of molds are brought together and the molten plastic injected. Each cable tie mold on the metal plate has a series of numbers and letters. Often, the molds are in numerical order and the highest number is the maximum number of cable ties that can be formed from one batch on that machine. The molten plastic is then ejected from the mold using pins.<sup>1</sup> Due to the nature of the manufacturing process, class and individual characteristics are created.

Class characteristics of the cable ties include general dimensions, color, and the mold number/letter combination impressions. To document all class characteristics for future use and comparison, cable ties were purchased from various national hardware stores. To start the database, a total of 29 bags of cable ties were purchased from Lowe's®, Home Depot®, and Harbor Freight®. From each store, 8", 11", and 14/15" cable ties were purchased. Ten cable ties were randomly selected from each store and size for examination. Twenty-five areas were selected to be measured or observed from different areas of the cable tie, including the clasp, body, and tail. These measurements and observations were made using a calibrated digital caliper and a stereomicroscope. The measurements were averaged over the sampled cable ties and a 95% confidence interval was calculated for each class characteristic.

Pin impressions from each manufacturing mold leave individual characteristic striation patterns on cable ties. When the plastic is ejected from the mold, striations can be imparted onto the malleable plastic. With larger quantity bags of cable ties, there are often multiple cable ties with the same mold number. Thus, cable ties with the same mold number impression can be compared to each other. To study how strongly the striation pattern matches between cable ties of the same mold and between ties of different molds, 12 bags of 100 cable ties were purchased from Nelco™ Cable Ties (Pembroke, MA). The 12 bags were from four separate lots. The first six bags were from the manufacturer Hua Wei Industrial (Taiwan) and were natural colored. The next six bags were black colored and from the manufacturer Kai Suh Enterprise (Taiwan). The majority of the bags had mold numbers in numerical order with multiple cable ties with the same mold number. The cable ties with the same mold number were compared to each other and had high amounts of matching striations whereas cable ties of different mold numbers had almost no matching striations. This indicates that matching striations are successfully transferred to cable ties with the same mold numbers.

Creating a cable tie database could be an extremely useful tool for forensic investigations. A database containing multiple measurements will yield a better chance for successful identification of brand/manufacturer. A large enough database may even provide an estimate to the likelihood of finding a particular cable tie from a random location, such as a crime scene. This study shows the potential evidentiary value of cable ties and should provide a practical reason for their collection by crime scene personnel and their subsequent examination in the laboratory.

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### Cable Ties, Database, Class and Individual Characteristic



## E65 “What's Wrong With Putting Crime Victims in Jail?”

Patricia C. Smith, MSL\*, Harris County District Attorney's Office, Houston, TX 77002

**Learning Overview:** The goal of this presentation is to bring awareness to the forensic science community and educate society about the importance of crime victim rights and ensuring crime victims are not revictimized during the legal process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing practical and educational tools to ensure crime victims and their feelings are a factor in the legal process and encourage interdisciplinary outreach to better treat and protect crime victims.

The United States Criminal Justice System is arguably the best system in the world; however, it has a tendency to let society down and get it wrong. The system has its flaws, but not always in the way people think. Society empathizes with crime victims, but arguably crime victims are the most ignored group within the Criminal Justice System.<sup>1</sup> However, the Criminal Justice System has helped and held those accountable more often than not. There are thousands of crime victim programs, advocacy and watchdog groups, and legislation tailored for crime victims. Yet, crime victims still continue to feel let down by a system that vows to protect and obtain justice for them.

In Texas, there are approximately 146 organizations in 94 cities that provide various types of crime victim support.<sup>2</sup> According to Domesticshelters.org, case management, resources, and referrals are the most common services utilized. The federal government developed crime victims' rights as outline in Title 18 U.S.C. section 3771, which states crime victims have a “right to be reasonably protected ... right to reasonable, accurate, and timely notice of any public proceeding, ... right to be reasonably heard at any public proceeding, and ... right to confer with the attorney for the Government in the case.”<sup>3</sup>

It's important for crime victims to know they have rights. Unfortunately, at both state and federal levels, crime victims appear to be the least aware of their rights.<sup>4,5</sup> Part of their rights are to not participate, regardless of the reason.<sup>6</sup> There are many cases where the victim recants, outright lies, or says they do not want to participate. The primary motivations were the victim did not want to be the one to send their loved one to jail, they just wanted the offending person to leave, or they were angry with that person.<sup>7,8</sup> The reasons a crime victim does not want to testify or participate in the process are important and should be taken into consideration.<sup>7,8</sup>

Prosecutors take crime victims seriously and generally want to know the “whys.” However, prosecutors will not stop communicating with the victim or continuing to prosecute the offender.<sup>9</sup> Typically, prosecutors will not force a victim to testify, but there have been instances where prosecutors felt there was necessity to put or keep an offender in jail, despite the desires of the victim.<sup>1,10</sup> Prosecutors across the nation and specifically, in Harris County, TX, have a legal right to detain a crime victim in order to ensure their presence in court.

Local and national cases will be examined.<sup>11-13</sup> Federal and state laws will be reviewed. It is important to include the psychological impact when a crime victim is detained and what the state can do to eliminate or lessen the emotional and sometimes physical damage.<sup>14</sup> Furthermore, there will be discussion of possible alternatives prosecutors can use to prevent the crime victim from feeling like the defendant and what society can do to improve their understanding and role in the Criminal Justice System. Finally, there will be a discussion of the repercussions for prosecutors.<sup>15</sup>

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### Crime Victims, Detention, Jail



### E66 Computed Tomography (CT) Scans and Autopsy Results of Nine Civilian Casualties of a Terrorist Attack

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**Learning Overview:** The goal of the presentation is to offer an overview of the injuries that can be found in victims of terrorist attacks and to explain how to integrate the autopic data, the information given by multi-planar reconstruction, and 3D volume-rendering techniques.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exposing the wide spectrum of features that can be found at the imaging and autopsy stages and demonstrating the importance of performing CT scans in order to provide accurate/full forensic autopsies in terrorist attack cases.

Prior to the rise of domestic terrorism, in many Western countries, pathologists experience of injuries caused by bombs and other particular types of unusual weapons, such as swords and unusual firearms, was limited to war casualties. In an Asian country, nine tourists were killed during a terrorist raid in a restaurant. The group used pipe bombs, machetes, and various types of firearms (handguns, submachine guns, and AK-47 assault rifles) loaded with illegal bullets. Local pathologists performed full forensic autopsies on the bodies. CT scans and new autopsies were performed on all nine victims. For the postmortem radiologic investigation, two techniques (multi-planar reconstruction and 3D volume-rendering) were used, obtaining both 2D images and 3D reconstructions. The first two cases presented slash wounds of the head and neck inflicted by machetes, while in the last seven cases, gunshot wounds were found (in two cases associated with injuries caused by explosive devices).

The terrorists used firearms that shot at different velocities: handguns, submachine guns, and AK-47s. One of the most important ballistic characteristics was the fact that prohibited ammunition (expansion or hollow point bullets and fragmenting bullets) were used. Expansion bullets are also called “hollow point,” because they collapse upon impact with hard surfaces such as bone, which increases and expands the kinetic energy transfer. Fragmenting bullets break into several pieces, which exponentially increases the injury area.

In general, CT is considered particularly useful in the imaging of terrorist attack victims, since it is highly sensitive in detecting firearm and blast injuries (in particular, fractures), and it can reliably detect glass or metallic fragments. In these cases, CT scans proved to be very useful to reconstruct the bullet track, revealing the bone and metal fragments deposited during cavitation. Moreover, in many of the described cases, death was caused by headshots. CT scans were able to identify entrance holes in the skulls (characterized by the typical conic shape). Moreover, in two cases, CT scans revealed miscellaneous objects identified as low-energy shrapnel released by Improvised Explosive Devices (IEDs). Shrapnel is oftentimes added to IEDs by terrorists to increase their destructive potential. Terrorists also consider location of the attack to enhance severity. In confined spaces, the energy released by explosions can be increased four-fold to eight-fold.

In conclusion, as demonstrated in these cases, casualties of terrorist attacks exhibit a huge spectrum of injuries whose characteristics are relatively unusual for those who practice forensic pathology in Europe. In victims presenting explosives or firearms injuries, multi-planar reconstruction and 3D volume-rendering proved to be highly valuable to understand the types of weapons used and the dynamics of the attack.

#### Terrorism, Military Weapons, Postmortem Imaging



### E67 Forensic Facial Reconstruction in Identification vs. Archaeological Investigation: Science or Art?

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how 3D forensic facial reconstruction in plastiline clay is conducted, using anatomical knowledge to identify and follow markers on the skull for recreating a face. It has been argued that forensic art is a subjective rather than objective endeavor and employs artistic license more than science. While it is true that there are areas of subjective artistic interpretation involved, forensic facial reconstruction is capable of nearly 70% accuracy in the hands of a well-trained forensic artist and when executed within acknowledged scientific guidelines.<sup>1</sup> Healed bone from facial injuries or bone loss from dental problems contribute unique markers to identifiability. Attendees will come to understand how scientific guidelines and methods for “reading” a skull are based upon evidence of musculature, activity, and ethnicity that all contribute to craniofacial morphology. Measurements based upon data sets of specific populations and historical periods are used to attain as much accuracy as possible. Conversely, attendees will also learn where and when artistic license is necessary for those areas of the skull that do not provide information about facial features. Attendees will learn the difference in goals of forensic facial reconstruction in aiding identification versus that of forensic reconstructions applied toward archaeological investigations to bring images of past peoples to life.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community by demonstrating and promoting the value of forensic facial reconstruction artists who are also forensic anthropologists or have access to them for interpretation of facial structures. Forensic anthropologists trained in forensic art are uncommon but not unheard of. Nuanced cues for musculature attachments to facial bones are easy to miss for the untrained eye, as well as the ability to differentiate bone trauma remodeling from trauma that contributed to death or incurred postmortem. Forensic facial reconstruction in conjunction with forensic anthropology creates specialty skills that go beyond artistic interpretation. It is a goal of this presentation to encourage greater understanding and appreciation for those skills and dispel the misunderstanding that forensic facial reconstruction is primarily “art” over science.

This presentation will demonstrate that forensic art benefits most from the artist who is also a forensic anthropologist. The value of this in-depth knowledge toward determining the proper approach to each facial reconstruction, whether in a purely forensic context or for historical and archaeological purposes, cannot be overstated. Misinformation about the deceased provided to forensic artists leads to inaccurate reconstructions. Forensic art in combination with anthropological knowledge can offer unique value toward reconstructing images that aid in identification and visual understanding of human beings past and present.

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**Forensic Facial Reconstruction, Facial Anatomy, Archaeology**



### E68 Clinical Implications of Using Alternate Light to Assess Bruises

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**Learning Overview:** At the end of this research-based session, attendees will be able to identify the benefits and challenges of incorporating alternate light into the clinical assessment of cutaneous bruises in living subjects.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how victims of violence often have cutaneous bruises that are challenging to see when subtle, when found on older persons, and when found on persons of darker skin tones. Using an Alternate Light Source (ALS) in clinical settings can provide findings in support of a history of new blunt force trauma and weeks-old trauma.

After attending this presentation, attendees will identify the efficacy of using alternate light as an adjunct in the clinical assessment of blunt force trauma. Violence victims are often subjected to blunt force trauma that, due to skin color/pigmentation and injury age, can result in cutaneous bruises that are latent or barely visible. Additionally, family violence victims across the life cycle often receive serial physical abuse resulting in contusions of different ages. Failing to identify bruises can result in legal and health care treatment disparities.

In a recently completed longitudinal, randomized controlled study, an ALS found intentionally created upper and lower arm bruises were better visualized at 415nm to 450nm with yellow and orange filters when still visible in white (ambient) light and when no longer visible to the naked eye. Test subjects were assessed up to 21 times over 30 days. The findings were statistically significant in subjects from six known skin tones (dark, brown, tan, intermediate, light, and very light).

The findings from this study strongly support the use of alternate light in clinical settings to assist in the assessment of reported blunt force trauma in the presence of visible bruises in white light and bruises no longer visible in white light. However, alternate light absorption is not pathognomonic for the presence of current or recent bleeding from bruises under the skin surface. This presentation will trace prior research of using colorimetry alternate light in the assessment of bruises, topical products, and other naturally occurring skin artifacts that could mimic bruises. Attendees will learn to differentiate ALS findings that fluoresce versus absorb light and why escaped blood under the skin will absorb light.

The use of an ALS in a clinical setting can be used to support the consistency of the location of blunt force trauma obtained per patient history and to further support finding on a physical examination, such as pain or tenderness at the site(s) of absorption. Photographs of bruises under different alternate light wavelengths will be presented as well as clinical tips on taking photographs of ALS findings in a darkened exam room.

This presentation will discuss future bench and clinical research needs based on the study's findings. For example, in this study, researchers used yellow and orange goggles with plastic lenses sold and distributed by the ALS manufacturer. However, using a colorimeter, there was inconsistency in the measured values of all the yellow and orange goggles. In addition, other companies sell yellow and orange goggles that also vary in color from one set of goggles to another. Future research is needed on assault victims, especially those of darker skin tones, who report manual strangulation.

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#### ALS, Bruises, Absorption





## E69 Ocular Measures in the Detection of Deception

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**Learning Overview:** After attending this presentation, attendees will understand the principals behind the use of eye movement tracking, pupil diameter changes, blinks, and other oculometric parameters as measures of deception.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about alternative physiological measures that can be used in place of, or in conjunction with, traditional polygraph measures, along with data on their effectiveness. Current state-of-the-art commercial systems that employ these methods will be reviewed.

Law enforcement, military, and governmental agencies need robust credibility assessment and deception detection tools to keep up with an increasingly large number of investigative cases. Humans historically have shown poor performance when performing this task without aid. Polygraphy has been the predominant method of credibility assessment since it was first used by law enforcement in the 1920s. While this methodology can claim a high degree of accuracy when properly administered and used to investigate specific instances of events, it has proven to be less than optimal in several situations, leading to the investigation of alternative techniques and technologies. The National Research Council of the National Academies of Sciences has noted: *The limitations of the polygraph, especially for security screening, justify efforts to look more broadly for effective tools for deterring and detecting security violations.*<sup>1</sup>

Polygraphy hypothesizes that individuals in deceptive situations experience an emotional response that causes arousal of the Autonomic Nervous System (ANS). Increase in arousal is detected through measurement of changes in physiological functions in respiration, blood pressure, and skin conductance. Change in the responses of cognitive and perceptual processes can be measured and employed in a similar manner. Changes in eye movement patterns can serve as an indirect measure of memory and effectively indicate concealed knowledge. Like polygraph measures, pupil diameter is affected by arousal in the ANS but also by cognitive workload through the Central Nervous System (CNS). This provides the potential to combine CNS and ANS measures to investigate prior knowledge and deception detection. Similarly, research has shown that blink parameters are diagnostic in determination of deception, with the differences accounted for by both theories of cognitive load as well as arousal-based theories.

Ocular measures of veracity have applicability across a variety of domains. Determination of concealed knowledge and credibility assessment are obvious applications for use in law enforcement and suspect questioning. Source verification is another application well suited to this technique as it permits a quick screening of an individual to determine if other credibility assessment methods should also be employed. The various protocols can be applied in other areas including customs, border crossing, and questioning of enemy combatants.

As this technology moves from research to commercial products, this report will provide a background to both better understand the potential of such applications as well as determine their effectiveness for specific situations. After presenting information on the principles and practicalities of collecting ocular measures, an overview of currently available commercial systems will be provided. Attendees will leave with a better understanding of the benefits and limitations of this approach and how it compares with current deception detection methodologies.

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### Deception Detection, Polygraph, Ocular Measures



## E70 Breaking the Code to Better Understanding Asian Youth Gangs

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**Learning Overview:** The goals of this presentation are to present timely data on Asian Pacific Islander youth gangs and offer strategies on how to recognize and interpret various tattoos and graffiti associated with these gangs, which could assist the medical examiner/coroner and death investigator in the positive identification of the decedent out in the field and/or in the autopsy room. In addition, this presentation will also discuss some of the recent gang enhancement laws that California, North Carolina, Virginia, and Idaho have in place to help tackle this deadly problem.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering new strategies to help keep medical examiners/coroners, death investigators, law enforcement officers, and first responders safe when dealing with these gang members so everyone can go home safe at the end of their shift.

Every day we hear of someone who has fallen victim to gang violence. Youth gangs throughout the United States continue to terrorize the neighborhoods they claim as their own, causing citizens in these gang-infested neighborhoods to live in constant fear of their lives. As a result of the recent influx of gang violence and gang-related homicides in all communities, the safety of first responders and investigators at crime scenes are jeopardized. This leaves medical examiners/coroners, death investigators, and detectives as possible targets of intramural shootings because they are at the scene. Crime scene personnel shootings have increased nearly 55% in the past year. Throughout the United States, gang violence has risen over 65% in the past 12 months. Every state has gangs, and the problem is getting much worse in areas that traditionally do not have gang problems. Gangs are not just an urban problem, but a suburban and rural problem as well.

With the population of Asian and Pacific Islander Americans (API) continuing to rise in the United States, so do their needs. Unfortunately, not all Asian Americans are as uniformly educated, acculturated, and financially stable as the myth of the “model minority” suggests. Although adults from the API region have adapted well to life in the United States, serious problems have emerged among Asian American youth. In particular, youth gang violence in the API community has dramatically increased in the past few years by nearly 85% nationwide according to the United States Department of Justice, Office of Juvenile Justice and Delinquency Prevention. In Los Angeles County, CA, there are currently 275 Asian youth gangs, with a total gang membership of over 12,000. In neighboring Orange County, CA, gang involvement has reached an all-time high with more than 87 documented gangs and a membership of more than 4,000. Demographics show gang members’ (male and female) average age is 15 years with a range of 8–22 years. Even more disturbing is the increase of Asian females involved in gang activity. In Orange County, where the Asian gang population makes up 18% of all gang members, there are 225 Asian female gang members, up 78% from last year. Other surrounding counties in California and the cities of Philadelphia, PA, Portland, OR, and Fairfax County, VA, have seen similar trends with the rise of Asian youth gangs. For this study, more than 700 gang members were interviewed in the streets, jails, and juvenile halls in California, Virginia, and Pennsylvania. This study identified ten distinct manifestations of gang violence and eight ethnic differences and similarities among Asian gangs (i.e., definite cultural differences between Asian gangs and various other ethnic gangs; drugs; weaponry; killing over turf/territory; extortion; defacing property/graffiti; women in gangs).

This study found ethnic characteristics of the Asian street gang are different from other ethnic street gang (African American or Latino). The primary motivation of the Asian gang is monetary profit. Asian gangs tend to be extremely violent, prey on their own culture, and are extremely mobile, meaning that they move from one geographic area to another to commit crimes or evade law enforcement. What makes this study unique is that this study has identified and categorized Asian gangs as either “traditional” or “non-traditional” in their motivations and crimes that they commit.

One group of Asian gangs is the “Pacific Islander” gangs. The Pacific Islanders are a category comprised of Filipinos, Samoans, and Chamorros, who will often follow the “traditional” patterns of gang behavior. In essence, they are “traditional” in the sense that they model themselves from the Latino and African American gangs.

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### Asian Gangs, Gang Enhancement Laws, Youth Violence



## E71 Characteristic Injuries of Organized Dogfighting

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**Learning Overview:** After attending this presentation, attendees will be able to identify dog breeds commonly utilized for animal fighting, recognize the pattern of injuries associated with organized dogfighting, and how to appropriately document dogs suspected of being exploited in the commission of this crime.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the recognition of this clandestine crime. Animal fighting, such as organized dogfighting, represents intentional, severe abuse of animals. Animal fighting is illegal in all 50 states and the federal Animal Welfare Act prohibits animal fighting ventures. Still, this organized abuse of animals is rampant throughout the United States. Organized crimes, such as racketeering, illegal gambling, illicit drugs, and firearms offenses, are frequently associated with animal fighting. Therefore, it is critical that law enforcement possess a basic understanding regarding the animals involved, the pattern of injuries associated with organized animal fighting, and how to appropriately document these animals.

Often, veterinarians are requested by law enforcement to examine animals alleged to have been utilized in organized animal fighting. In such cases, the veterinarian must be able to identify, collect, and properly preserve evidence found on the animal(s). Though veterinarians are typically unfamiliar with the pattern of injuries associated with this crime and how to properly handle evidence, this presentation will further serve to assist veterinarians in recognizing, responding to, and assisting law enforcement in the investigation of animal fighting, while highlighting the most current research and techniques.

In the United States, higher-level (professional and hobbyist) fighting dogs are almost exclusively American Pit Bull Terriers. Research by Intarapanich et al. compared the distribution and extent (number of body zones affected) of injuries observed in Pit Bull-type dogs seized in conjunction with organized dogfighting to dogs of various breeds of the same sex and similar weight involved in spontaneous dogfights.<sup>1</sup> The five most commonly injured areas associated with organized dogfighting, in decreasing order of frequency, were the front legs, dorsal and lateral aspects of the head, muzzle and oral mucosa, dorsal and lateral neck, and ventral neck and chest. The most commonly injured areas associated with the spontaneous dog fights, in decreasing order of frequency, were the pinna followed by the dorsal and lateral neck as well as the front legs. Looking closely at the percentage of dogs in each group that sustained injury, a clear difference was appreciated, as dogs engaged in organized dogfighting were much more likely to present with multiple injuries in these areas, as opposed to those engaged in spontaneous dog fights, where there were only single or limited injuries. Therefore, when prevalence was considered along with the most commonly injured areas, there was a distinct difference in the pattern of injury between spontaneous and organized dogfighting.<sup>1</sup>

The distribution of organized dogfighting injuries were further validated by Miller et al., who found that scarring and wounds associated with organized dogfighting were primarily concentrated on the front legs, dorsal and lateral head, and muzzle and oral mucosa.<sup>2</sup> Additionally, it was found that dogs with ten or more injuries to the front legs, dorsal and lateral head, and the muzzle and oral mucosa to be a reasonable threshold for high risk of dog aggression, especially in male dogs.<sup>2</sup> Therefore, when documenting medical findings it is of the utmost importance that all wounds, scars, and lack thereof be thoroughly documented and photographed. A “scar chart” or wound-and-scar body diagram should be completed to demonstrate the distribution of scars and/or wounds. However, typically not all dogs in a dogfighting yard will have wounds and/or scars present. Some dogs may simply be too young to have yet been fought, while others may be utilized primarily for breeding purposes or may be maintained to act as a guard dogs or may serve as pets.

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### Dogfighting, Animal Abuse, Veterinary Forensics



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### E72 The Use of Forensic Osteology in Animal Cruelty Investigations

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**Learning Overview:** The goal of this presentation is to inform attendees of the value of forensic osteology analysis in animal cruelty investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating that the use of forensic osteology in cases of animal cruelty may not only offer more specific information than radiographs, but also how this information could be used to file additional charges against the abuser.

Forensic anthropology is a common discipline utilized in the identification of human remains that are decomposed or otherwise unrecognizable. Unfortunately, it is not possible to glean all the same information from animal bones as is possible with a forensic anthropological analysis of human skeletal remains. For example, ancestry/breed and sex are often indeterminable. These factors, in addition to the technical definition of anthropology (the study of man), make the term forensic anthropology inappropriate in an application to animals. Therefore, the phrase forensic osteology is recommended when referring to the analysis of non-human skeletal remains. Applied to both humans and non-humans, forensic osteology is the study of skeletal remains to determine the causes and circumstances of death. In addition to developing in the same manner as human bones, animal bones also respond to trauma in generally the same manner. While there are structural and morphological differences (such as the shape of the cranium) that result in different effects, how bones react under force is generally predictable, making it possible to employ similar practices to the analysis of trauma in animals as are utilized for human victims.

The discipline of forensic osteology is not generally utilized in the investigation of animal cruelty crimes. The forensic examination of deceased animals is performed by veterinarians who typically rely on radiographs or Computed Tomography (CT) scans to visualize the skeleton. Although it is possible for injuries to not be visible through imaging, many veterinarians do not have the time, resources, equipment, or training to process deceased remains down to just skeletal elements. Similarly, veterinarians are trained to analyze skeletal trauma in regard to fixing it—not as it applies to a cause of death. This lapse in analysis occurs despite the results of a survey that was conducted of prosecutors and indicated that “bones” ranked higher than DNA when survey participants were asked what types of evidence from the scene influenced their decision(s) to prosecute animal cruelty crimes.

This presentation will discuss two specific animal cruelty cases for which the discipline of forensic osteology was utilized. Both victims to be discussed were dogs, with one death the result of multiple gunshot wounds and the other related to blunt force injuries. In both cases, the osteological analyses were able to offer additional and more specific information than the radiographs. In one case, this additional information resulted in an additional felony animal cruelty charge. Finally, there will be discussion of the osteological findings that have been found to be suggestive of dogfighting.

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#### Animals, Cruelty, Osteology



## E73 Gap Assessment of Stress, Vicarious Trauma, and Resiliency for Forensic Science Professionals

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**Learning Overview:** After attending this presentation, attendees will understand: (1) the unique characteristics of vicarious trauma experienced by Forensic Science Professionals (FSPs); (2) factors correlated with stress and burnout among these professionals; and (3) recommendations for improved resiliency.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science and medicolegal death investigation communities by addressing the gap in scientific literature related to stress, vicarious trauma, and burnout for FSPs. Attendees will learn the results of a recent study that used the Vicarious Trauma-Organizational Readiness Guide (VT-ORG) developed by the Office for Victims of Crime (OVC) modified for FSPs. Vicarious Trauma (VT) has been characterized by intrusive thoughts, avoidance and withdrawal, and symptoms of tension and disturbed sleep.<sup>1</sup> Responses are related to the indirect exposure to a traumatic event through second-hand materials, such as images or stories. Professionals suffering from these symptoms report lower job satisfaction, emotional exhaustion, increased thoughts of leaving their position, and stress in their family lives.<sup>2</sup> Similarly, burnout is characterized by fatigue, poor sleep, headaches, anxiety, irritability, depression, hopelessness, cynicism, and a lack of professional efficacy.<sup>3</sup> Burnout and VT often develop together and many of the symptoms overlap. Attendees will learn suggestions for future research and policy implications regarding resiliency resources.

A subset of VT literature has documented a litany of issues in first responders, law enforcement, legal professionals, and human services providers.<sup>4-6</sup> A handful of studies have attempted to characterize stress responses and identify effective coping strategies in FSPs. One study of crime scene investigators' physical responses to stress found that heart rates increased by approximately 50 beats per minute at crime scenes compared to routine activities.<sup>7</sup> A 2011 study found that although virtually all of the digital investigators who participated were satisfied with their jobs, 68% felt they were under a lot of stress and half stated that aspects of the job made them upset.<sup>8</sup>

Studies with first responders, such as police, firefighters, and emergency medical services, have supported several strategies for addressing stress and increasing resiliency. Resiliency is the ability of individuals exposed to highly disruptive events to maintain both healthy psychological and physical functioning. Studies of effective interventions have concluded that organizations must train employees to accept and recognize stress as a routine part of the job and offer them strategies to reduce the stress.<sup>9</sup>

The current study used an anonymous, online survey of seven laboratories to ask FSPs about their self-reported stress levels and their perception of organizational support for stress reduction. The first instrument, the VT-ORG, modified for FSPs, gathered information about the perception of the organization's efforts to support this stress in employees. The second survey, Professional Quality of Life (ProQOL), contained self-reported questions about the employee's stress responses and job satisfaction. Additionally, a demographic questionnaire asked questions relevant to job stressors. The results will be fully discussed as well as suggestions for future research and policy implications regarding resiliency resources for FSPs.

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### Forensic Science Professionals, Vicarious Trauma, Stress



## E74 Advances in Color and Texture Analysis of Human Hair

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**Learning Overview:** After attending this presentation, attendees will understand that color and texture of hair samples can be quantified to support the classification of hair features in forensic analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing methods to quantifying these features and various statistical techniques that can be used to classify different hair samples.

Hairs and fibers are very common types of trace evidence found in crime scenes. Hairs in particular are naturally shed by most mammals, giving them a higher transfer potential at a crime scene. The first stage in the forensic analysis of hair evidence is the attempt to determine the source of the hair and whether the hair is animal or human. Animal hairs contain significantly different features when compared to human hair and, in some cases, the animal species may be determined. As for human hair, it contains ancestral differences such as color, texture, cross-sectional shape, shaft form, and others that can aid in suspect identification. The analysis of human hair can sometimes be problematic due to the subjectivity of the descriptors used. Broad categorical descriptors derived from the examiner's skills and experience are sometimes used to classify different features, and these may vary between examiner. Also, there exists the potential for experimenter bias, which can be mitigated through the use of empirical data and more objective/quantifiable descriptors.

Forensic hair analysts evaluate features observed at both macroscopic and microscopic levels. Macroscopic features are those that can be easily seen by the naked eye, such as length, color, and hair form. Microscopic features evaluate the three main areas of the hair: the cuticle, the cortex, and the medulla. Each of the areas contain features that can be used to associate or discriminate between two samples. The cortex contains many characteristics that can be used for comparison (e.g., the texture and the color of the hair). However, when analyzing these two features, subjective and relative descriptors are usually used to describe them. Efforts have been made to empirically determine color with some success; however, little has been conducted to analyze the texture. Development of high-resolution digital microscopes allows researchers to closely examine these characteristics and obtain more accurate measurements. In this study, a VHX 6000 Keyence® digital microscope was used to explore the variance within the color and texture characteristics throughout the hair, as well as the inter- and intra-person variability. The color distribution of hair and texture analysis were obtained using MATLAB® image software. The samples consisted of 25 fallen hairs from 75 individuals of different ancestries between the ages of 18 and 35 years. Only samples with natural hair color were considered for this study. Analysis of variance was performed to assess the inter- and intra-sample variability among hairs. The color distribution of the hair was determined using a Red-Green-Blue (RGB) color model. This enables a more accurate description of the color of the hair given that it encompasses the natural variability of color change through the hair, allowing for a more comprehensive assessment. Clear differences can be seen in the distribution between the most common hair colors (black, brown, red, and blond). As for texture analysis, neural networks were used to attempt to classify the texture of hair based on grayscale images, allowing for a more objective analysis of texture.

This presentation will increase the knowledge of the variability within hair features and provide a novel method for quantitatively assessing color and texture of human hair. It is important to note that this study is aimed at supporting the forensic hair community by providing a tool to quantify features to classify unknown and known hair samples, not necessarily to compare hair samples directly.

### Hair Analysis, Macroscopic Measurement, Neural Networks



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### E75 Discrimination Between Human and Animal Blood by Attenuated Total Reflection/Fourier Transform Infrared (ATR/FTIR) Spectroscopy for Forensic Purposes

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**Learning Overview:** After attending this presentation, attendees will gain knowledge about: (1) the limitations of current methods for bloodstain analysis during forensic investigation; (2) the importance of species identification from blood traces found at a crime scene; (3) the significance of a non-destructive method for examination of trace evidence at a crime scene; and (4) the advantages of FTIR spectroscopy in forensic investigation. The goal of this presentation is to disseminate results of FTIR spectroscopy for bloodstain examination as well as the results of chemometrics for distinguishing between human and animal blood.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by disseminating results of the non-destructive and rapid discrimination between human and animal blood at a crime scene immediately after discovering a crime scene.

Bloodstain identification is one of the most essential aspects of forensic casework involving violence. Presumptive tests can be used for unveiling the presence of blood. Unfortunately, false positive results may be obtained from substances other than blood. Subsequent DNA profiling can be performed to compare the profile with those in a DNA database. This can waste time, money, and other resources if the DNA was obtained from a stain of non-human origin, in cases where human DNA was of intention. Therefore, another very important step of forensic casework is the identification of origin of a stain: human or non-human blood. Discrimination between species can be especially critical in hit-and-run incidents for confirming suspect's testimony whether an animal or a human was involved in an event. Therefore, having a reliable method for non-destructive species identification from a bloodstain would be extremely beneficial and would save time, money, and other resources.

This study proposes the use of ATR/FTIR spectroscopy for identification and characterization of bloodstains. Specifically, this technique in combination with advanced statistical analysis, showed promise for differentiating between human and non-human blood traces from a pool of forensically relevant species. The statistical modeling demonstrated high prediction accuracy of bloodstains from unknown samples. The great advantage of this technique is its non-destructive nature, which makes it practical for forensic applications. The non-destructive examination can be performed on the same sample using ATR/FTIR spectroscopy and subsequent DNA analysis. Commercially available portable instruments make on-scene examination possible. Rapidly obtained results on scene give the great benefit of saving time, money, and other resources combined with immediate results during the first, critical hours of a criminal investigation.

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#### Bloodstains, Human Origin, Non-Destructive Analysis



## E76 Linking Footprints to Feet: Research Advances and a *Daubert* Case Study

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**Learning Overview:** After attending this presentation, attendees will learn the current scientific foundations and limitations of utilizing a footprint or footprints found at crime scenes to identify the criminal.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight and knowledge into the relatively new field of forensic podiatry, with a focus on footprint impression analysis.

Footprints recovered from crime scenes do not always display papillary ridge marks like those of fingerprints and, in these situations, footprint analysis involves measurement and comparison of the footprint's outline, shape, and consideration of other features.<sup>1</sup> The discriminatory and individualistic value of bare footprints is well-established, as are their use in linking (and unlinking) suspects to crime scenes. Research has found the odds of a chance match of a bare footprint to be one in 1.27 billion.<sup>2</sup> Numerous factors contribute to this individualistic nature of footprints, including a person's biomechanics, anatomy, weight, footwear, and particular habits or activities.

While bare footprint individuality has been proven, research on sock-clad footprints and foot impressions in insoles has been sparse. This research will present findings from a study published on the analysis of sock-clad footprints that established similar individuality to bare footprints. There will also be discussion on initial findings from a recently completed study on foot impressions in shoes.<sup>3</sup> The ability to establish the individuality of a footprint, whether bare, sock-clad, or inside footwear, has important consequences for forensic experts comparing suspects to such impressions.

Further, previously numerous methods of footprint measurement and comparison were proposed, some utilizing descriptive statistics to establish their validity. A method, tested with inferential statistics, was recently advanced by Reel et al. and has been found to be highly reliable across a combination of reliability tests.<sup>4</sup> This method, known as the "Reel Method," is now considered by some experts to be the gold standard for determining 2D linear footprint measurements for forensic comparison.

The state of the science of forensic footprint analysis will be reviewed with particular emphasis on meeting the *Daubert* standard, which was met in an analysis of a bloody sock-clad footprint found at a homicide scene. The case study of the *Daubert* challenge will be reviewed in the context of the latest scientific advances. Limitations remain in the analysis and comparison of footprint evidence and the presentation will highlight research that remains to be done as well as the aspects that can be relied on to link (or unlink) a footprint found at a crime scene to a suspect.

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### Footprint, Footprint Analysis, Foot Impression





### E77 A Tale of a White-Tailed Deer: Anomalous Serology and DNA Results Offer Clues in an Alleged Hit-and-Run Case

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**Learning Overview:** After attending this presentation, attendees will be familiar with certain types of biological evidence that can be encountered in hit-and-run cases and the potential for erroneous identification of evidence using DNA and serology testing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that forensic analysis of biological evidence in hit-and-run cases can lead to false conclusions and underscores the importance of integrating wildlife testing as a critical component in forensic identification.

Serious bodily injury or loss of life can result from an accidental or intentional impact between a motor vehicle and a pedestrian. In cases involving allegations of hit-and-run, the recovery and testing of biological evidence can assist in advancing investigative efforts and incident reconstruction. Testing for the presence of blood can become the driving force in the pursuit of the offending vehicle and ultimately the suspect. Presumptive tests for blood, such as the phenolphthalein-based Kastle-Meyer test, the Hemastix<sup>®</sup> test, and the luminol/Bluestar<sup>®</sup> test are easy to use and are relatively inexpensive to screen samples for the possible presence of blood. However, those tests are not specific to human blood and can result in false positive reactions to substances other than blood. The tests will react to hemoglobin-containing blood from any mammal as well as blood from other organisms. Using both the Kastle-Meyer test and the Bluestar<sup>®</sup> test, this study observed positive reactions to blood samples collected from birds, reptiles, amphibians, and bony fish. Also observed were positive reactions to samples collected from circulatory fluids of many invertebrates, such as annelids (earthworms), arthropods (spiders), and others. The circulatory systems of those organisms contain iron-rich hemoglobin or copper-rich hemocyanin for respiration. Prudence is warranted in general when testing suspicious stains or biological samples on motor vehicles with presumptive tests for blood. The opportunity exists for encountering stains from various wildlife species, thus potentially leading to false conclusions. To illustrate this issue, an actual case study with complex parameters involving two vehicles and an allegation of a hit-and-run scenario will be discussed.

A female decedent was killed after being struck by a motor vehicle. The case parameters indicated another vehicle, which initially fled the scene. Examination of that vehicle revealed the presence of suspected biological material that was pursued as evidence of involvement in the same incident. Ultimately, the investigation focused on the second vehicle and the driver was charged with homicide because, among other reasons, biological tissue that appeared to be of human origin was found adhered to the undercarriage of his vehicle. Samples from the biological tissue were tested and revealed positive results with the presumptive Kastle-Meyer blood test. Additional confirmatory tests for blood using the RSID<sup>™</sup> blood card revealed negative results. DNA analyses using the Identifiler<sup>®</sup> Human Identification typing kit were unsuccessful in developing a DNA profile from the tissue samples. The failed DNA testing was initially blamed on sample degradation. However, close examination of the profile data revealed the presence of an allelic peak near the amelogenin marker that has been previously described as an artifact of animal DNA amplification with the amelogenin locus primers.

Testing for wildlife DNA is a relatively simple genetic assay using Polymerase Chain Reaction (PCR) technology. It targets a portion of the mitochondrial DNA (mtDNA) commonly used for population studies of various species. Population genetics has provided ample material for comparison of sequence data to determine species from unknown samples. The tissue samples in this case were tested for DNA from a wildlife species. A small piece of the tissue was removed using a sterile scalpel. The sample was then extracted using a QIAGEN<sup>®</sup> DNA Investigator Kit. The extracted DNA was quantified and PCR amplified using primers that target the cytochrome b portion of the mtDNA. The resulting sequence was compared to all sequences contained in<sup>®</sup> via the Basic Local Alignment Search Tool (BLAST). In this case, the sequence aligned with *Odocoileus virginianus* (white-tailed deer) and not *Homo sapiens*. The results confirm the need to keep an open mind about the source of biological material that is adhered to a vehicle. In this case, the biological material found on the undercarriage of the suspect vehicle was most likely picked up when that vehicle ran over the remains of a deer.

#### DNA and Serology, Hit-and-Run, False Positive



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### E78 Digital Photography of Decomposed Fingertips for Postmortem Fingerprint Identification

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**Learning Overview:** After attending this presentation, attendees will understand a method using digital photography and enhancing software to obtain postmortem fingerprints of decomposed decedents who are otherwise unidentifiable and traditional ink/digital fingerprint methods are ineffective.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a cost-effective and non-destructive postmortem digital photography fingerprint method to identify decomposed decedents in the medical examiner field.

In many medical examiner offices, the primary means of decedent identification is through fingerprints using ink or digital technologies. However, some decedents are too decomposed to utilize these technologies. When a decedent is desiccated or macerated, the fingertips are often dried, hard, and wrinkled or too moist to use standard ink/digital methods. In these cases, medical examiner offices must rely on alternate identification methods, such as radiograph/dental comparison, fingertip rehydration techniques, or DNA. These alternate methods can be labor intensive, costly, and destructive, and at times are ineffective. Radiograph/dental comparison and DNA often require the decedent to be tentatively identified. As a result, a method for capturing fingerprints of desiccated and macerated decedents is needed.

An improved method using digital photography and enhancing software, Adobe® Photoshop®, was developed to obtain suitable fingerprints from decomposed bodies for identification. The method requires a digital single-lens reflex camera, a macro-enabled lens, a tri-pod, and a suitable light source to photograph each fingertip. Each fingertip is photographed with a one-inch ruler held in plane with the fingertip. The ruler must be labeled to identify the fingertip (e.g., RI, RII, etc.) and ideally the case number. All images are captured in JPEG, for viewing purposes and RAW file formats. The RAW images are imported into Adobe® Photoshop® and are adjusted to mimic a fingertip that has been captured with ink. The workflow includes converting the image to black and white, rotating the image to the correct position, inverting the image so the ridge detail is black, flipping the image, and adjusting the image to a 1:1 ratio using the ruler. Minor non-destructive enhancing techniques, such as contrast, brightness, and dodging/burning, are used to enhance the ridge detail further. Each image is placed onto the corresponding location on a digital ten-print card and the card is submitted digitally to the Federal Bureau of Investigation (FBI) for analysis.

The method has resulted in the positive identification of several decedents. Ten-print cards have been successfully entered into the Automated Fingerprint Identification System (AFIS) by the FBI Special Processing Center as well as analyzed by the Latent Print Unit. Several tips have been learned during method development that ensures the best possible fingerprint image is captured. These case studies and lessons learned will be presented.

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#### Fingerprints, Photography, Postmortem Decomposition



## E79 Development of Baseline Performance Levels for Standardized Field Sobriety Tests in Sober Individuals

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**Learning Overview:** After attending this presentation, attendees will better understand the standard procedures used for the administration of field sobriety tests and the validity of the Horizontal Gaze Nystagmus (HGN), Walk And Turn (WAT), and One Leg Stand (OLS) tests at assessing impairment through the development of baseline performance levels in sober individuals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by examining how sober individuals perform on the Standardized Field Sobriety Test (SFST) to determine if the currently used battery of tests is fit for its purpose of identifying an impaired driver.

According to the Substance Abuse and Mental Health Services Administration (SAMHSA), in 2013, approximately 28.7 million people admitted driving while under the influence of alcohol at some point during the year.<sup>1</sup> One of the ways law enforcement has chosen to combat this problem is through the use of observations and qualitative tests to determine if a driver could potentially be impaired. The qualitative tests have become known as the SFST battery. The three components of the official SFST battery include HGN, WAT, and OLS. Law enforcement officers began to use the National Highway Traffic Safety Administration's (NHTSA) SFST battery in the 1970s, and they went into widespread use in the 1980s.<sup>2-3</sup> The purpose of the three tests is to assist law enforcement officers in determining whether or not an individual suspected of driving while impaired by alcohol should be arrested. In this manner, SFSTs are used as a pre-arrest screen. Limited research exists regarding baseline performance levels on these tests in a sober population. Additionally, there are numerous factors that the current battery of tests does not take into account. This research project examined the prevalence of a number of these variables, including age, sleeping habits, medical/physical history, recreational drug and alcohol use, and caffeine and nicotine intake, to determine if any correlation could be found between specific physical or physiological factors and how one performs on the SFSTs.

This research project focused on using sober volunteers to develop baseline performance levels for each test to determine if the SFSTs are valid and fit for their purpose of identifying an impaired driver. The purpose was to determine if a sober population can successfully meet the current standards and criteria used by law enforcement when evaluating the SFSTs. Participants in this study completed a questionnaire describing their medical history and various lifestyle choices. After having completed the questionnaire, the SFSTs were administered by a certified SFST practitioner to each volunteer in a controlled, isolated classroom setting to prevent any external variables from affecting the results. A group of participants underwent a second round of testing in a parking lot located next to a relatively busy road. This setting included distractions that may be present during real-life, drunk driving traffic stops. A separate cohort of individuals was also tested under two conditions: sober and after consuming alcohol. After all of the tests were completed, the results were evaluated to determine if the currently used three components of the SFSTs are fit for their purpose or if they are affected by other variables that are not commonly associated with driving impairment.

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1. Substance Abuse and Mental Health Services Administration. Results from the 2013 National Survey on Drug Use and Health: Summary of national findings. *NSDUH Series H-48*, HHS Publication No. (SMA) 14-4863. Rockville, MD: Substance Abuse and Mental Health Services Administration, 2014.
2. Rubenzer S. The psychometrics and science of standardized field sobriety tests, part 1. *The Champion*. 2003 May:48.
3. Stuster J., Burns M. Validation of the standardized field sobriety test battery at BACs below 0.10 percent: Final report. *PsycEXTRA Dataset*. 1998.

### Standardized Field Sobriety Test, DUI, Sober



## E80 Statistical Evaluation of Latent Prints Developed by Oil Red O Solutions

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**Learning Overview:** This presentation will compare the relative quality of latent prints developed by the two most commonly promoted Oil Red O (ORO) solutions on numerous substrates and in sequence with other developmental techniques.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enabling latent print examiners to utilize the ORO solution that would deliver the best results when processing for latent prints on porous substrates.

While Physical Developer (PD) can develop latent prints on dry and previously wetted porous substrates, PD processing utilizes multiple components that are costly, can be destructive to paper, have a short shelf life, and are not suitable for all scenarios. Conversely, 1-[2,5-Dimethyl-4-(2,5-dimethylphenylazo)phenylazo]-2-naphthol, more commonly known as Oil Red O (ORO), is simpler, less expensive, non-destructive to paper, and has a much longer shelf life than pre-made PD solutions.

ORO has previously been suspended in either propylene glycol or with 0.23 M NaOH in 77% methanol by volume, hereafter referred to as ORO1 and ORO2, respectively.<sup>1,2</sup> The relative ability of these two solutions to develop latent prints has previously been evaluated to a limited extent. Also, ORO has been recommended to precede PD when used sequentially in series with other latent print processing techniques.<sup>2-3</sup> In a 2013 study, Frick, Fritz, Lewis, and Bronswijk contrarily noted that there was slightly better contrast when ORO was placed after PD, but still recommended PD follow ORO because of PD's "destructive potential."<sup>1</sup>

This research evaluated the two different ORO solutions on exemplars of both thermally sensitive and insensitive paper to determine which solution developed higher quality prints on each substrate for more than 20 donors. Prints developed by ORO solutions were benchmarked against those developed by PD and against those developed in sequence with 1, 2-indanedione and ninhydrin. Finally, the robustness of ORO solutions was tested using aged natural prints. Print quality was measured using the Universal Latent Workstation's (ULW) Latent Quality (LQ) Metrics overall clarity score as well as scoring by five trained latent print examiners using unenhanced grayscale images.

Overall, latent prints were sufficiently developed by both ORO solutions on most thermal and non-thermal paper exemplars. On one non-thermal paper, ORO2 provided equal or greater contrast and detail than on the thermal paper ( $p=0.01$  and  $<0.001$  as analyzed by both LQ Metric clarity and examiner scores, respectively). Contrastingly, ORO1 had equal or significantly ( $p < 0.05$  for half of the donors) better results on a thermal paper compared to the non-thermal paper. ORO2 typically produced higher quality prints than ORO1 for all substrates with only a few exceptions for some thermal papers. The relative quality of prints produced by both ORO solutions were typically of equal or higher quality than PD, except for a few donors for which the inverse was observed. Additionally, ORO was tested sequentially following 1,2 indanedione and ninhydrin, but preceding PD. Neither 1, 2-indanedione and ninhydrin inhibited ORO, but the resulting ORO prints were not of as high a quality as prints produced by only ORO. In summary, both ORO solutions developed latent prints of suitable quality for comparison, but ORO2 generally produced prints of higher contrast/clarity.

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### Reference(s):

1. Frick A., Fritz P., Lewis S., Van Bronswijk W. Sequencing of a modified oil red o development technique for the detection of latent fingermarks on paper surfaces. *J Forensic Ident.* 2013;63(4):369-385.
2. Beaudoin A. Oil Red O: Fingerprint development on a 21-year-old cold case. *J Forensic Ident.* 2011;61(1):50-59.
3. Rawji A., Beaudoin A. Oil Red O versus physical developer on wet papers: a comparative study. *J Forensic Ident.* 2006;56(1):33-55.

### Oil Red O Solutions, Latent Prints, Statistical Evaluation



### E81 Tracking Trends Through the Peer Review Process

Jessica LeCroy, BS\*, Forest Park, GA 30297

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the unique application of the peer review process at the United States Army Criminal Investigation Laboratory (USACIL) through a simultaneous technical and administrative review of the case file and report. Attendees will also receive insight on why this new approach was needed and how it was developed. Attendees will be provided an understanding of how issues and errors detected in the peer review process can be monitored for trends and will be provided a summary of some of the trends noted during an evaluation of data gathered during calendar year 2018.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring a new peer review approach in practice in Latent Print and Footwear/Tire Track-related requests by the USACIL and how the peer review process is being utilized to track trends in the application of procedures and the mitigation of errors.

In addition to the traditional elements of administrative and technical review, the new peer review approach utilizes a specially designed checklist to rank errors detected in casework. This ranking system provides valuable insight into the critical nature of the errors and to better understand the potential impact of the error to reported results if it had not been detected. The review is categorized into several key components to better track where in the examination process issues routinely occur. The data gathered through the checklist is evaluated to look for positive and negative trends in the application of procedures or errors made in an attempt to improve case quality output and enhance the review process as a whole. Tracking of these trends allows managers and policy makers to adjust policy and resources with data to support the changes and to potentially evaluate how to prevent further review findings. This process also allows reviewers to provide suggestions, which may not be explicitly required per policy, but provides better overall collaboration between case examiner and reviewer to develop a more coherent work product. Results from an evaluation of data from 2018 will be discussed, which highlight the typical errors made and the impact of these errors to the case.

*The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the United States Department of the Army or United States Department of Defense.*

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#### Peer Review, Trends, Latent Prints



## E82 Examining Drug-Related Deaths While in Custody: A Look Into the Role That Race Plays in Drug-Related Custody Deaths in Texas

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**Learning Overview:** After attending this presentation, attendees will better understand the correlation between deaths related to narcotics during custody in the state of Texas and race. The goal of this presentation is to introduce the concept of drug-related death occurrences during custody and present the data behind the association of race and specific type of drug found to contribute toward the cause of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing understanding of the nexus which exists between race and narcotics-related deaths of individuals in law enforcement custody. This presentation will also provide recommendations as to the necessity for thorough medical examinations of illicit drug-related deaths that occur in law enforcement custody.

Deaths of Americans in the custody of law enforcement is a widespread discussion being had in today's society. While much of the debate involves officer shootings during arrest or initial contact, drug-related deaths while in custody are a less-emphasized topic of discussion. The relationship between the race of the decedents and type of drug indicated as a cause of death by the medical examiner were tested. The results suggest that there exists a statistically significant difference between the race of the individual and type of drug associated with their death while in law enforcement custody.

As public scrutiny of law enforcement's treatment of minority citizens increases, empirical research in the fields of criminal justice and criminology increase as well. Primarily, these empirical studies seek to determine the association between citizen and police, which is most visible at the moment of initial confrontation or arrest. What is examined to a lesser extent are the occurrences between citizen and police after arrest. This report intends to examine occurrences of drug-related deaths of individuals in law enforcement custody. Specifically, it seeks to explore the relationship between race and type of narcotics that contribute to the cause of death to individuals in custody.

In understanding the current cannon of research associated with race and overdoses within custody, this report used official data gathered by the Texas Justice Initiative. The data is obtained through the mandatory reporting system between criminal justice agencies in the state of Texas and the Attorney General's Office when a death occurs in their custody. While this reporting has been in practice for decades, it was not until 2005 that it became streamlined and, in 2016, made open to the public. This study examines deaths linked to narcotic use while in law enforcement custody within the state of Texas for the period of 2015 through 2019.

Data analysis was conducted by examining the causes of death noted by medical examiners for each of the original deaths that occurred within this time frame ( $n=2,975$ ); only 3.5% ( $n=104$ ) listed a narcotic as a noted cause of death. The dataset breaks down Race by either Black, White, Hispanic, or Other. For the causes of death, this study only focused on narcotic names that were listed under the cause of death by the medical examiner. Statistical Package for the Social Sciences (SPSS), a multiple response report, is used in order to capture multiple narcotics listed in some subject's causes of death. Binary variables are created to reflect Cocaine, Methamphetamine, Amphetamine, and Other. This report then employed an uncertainty coefficient measure to determine the relationship between the race of the decedent and the form of narcotic noted as a cause of death by the medical examiner. While the Race and Drug type were too far from a uniform distribution to use chi-square, uncertainty coefficient is deployed, and the likelihood ratio between race and narcotics gave significant results.

The analysis of data conducted in this study indicates that there exists a relationship between the race of the decedent in custody and the type of narcotics associated with that individual's death. More specifically, the data finds that there exists a statistically significant relationship between Race and Cocaine ( $p=.022$ ) as well as Race and Methamphetamine ( $p=.002$ ). The results were unable to determine a significant correlation between Race and Amphetamine use and Other drug use.

The purpose of this analysis is to further the understanding of illicit drug-related deaths occurring to individuals who are placed under the supervision of a law enforcement agency. This report seeks to educate the public on the widespread occurrences of illicit drug-related deaths in custody, the types of drugs associated with these deaths, and demographics of the decedents.

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### Overdose, Criminal Justice, Custody



## E83 The Application of 3D Motion Capture in the Analysis of Doubtful Forensic Cases

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**Learning Overview:** After attending this presentation, attendees will know how 3D Motion Capture (MOCAP) could help forensic pathologists in recreating a crime scene closer to reality and how it can be considered very helpful in shedding light on murders cases, accidents, or suicides, making the dynamic of the events clearer.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility to help both prosecutors and detectives to understand complicated dynamics, becoming a form of judicial evidence in court. For these reasons, this presentation highlights how the correct use of the 3D MOCAP could become an essential instrument in crime investigations over the years.

During the past few years, forensic sciences have quickly developed the application of new information technologies for the reconstruction of crime scenes. The MOCAP is the recording of the movement of the human body for the immediate or deferred analysis through virtual reproduction. Presented is a virtual model created through 3D MOCAP. This method is useful for realizing a 3D video that reproduces the dynamics of the scene of doubtful forensic cases. The proposed model is not used routinely in forensics but through the analysis of these cases, its applicative utility is demonstrated. In particular, the forensic purpose is to compare the injuries analyzed at the autopsy or the inspection of the victim with the possible dynamics compatible with the environments in which the event occurred and testimonies. A judicial inspection was conducted and a planimetry of the apartment was requested for each case. The device used for the acquisition of movements was a stereophotogrammetric system, composed of infrared OptiTrack® cameras capable of capturing movements up to 100 frames per second (fps). Some objects have been modeled in 3D manually, taking into account the original measurements. Two actors mimicked the movements of the aggressor and the victim in cases 1, 2, and 3. In case 4, only one actor was used. The rendering of the frames was entrusted to Supermicro systems with multiprocessor Xeon quad and Nvidia Quadro Plex® systems for Graphics Processing Unit (GPU) rendering projects.

Reported are four forensic cases with different death modalities: two murders, an attempted murder, and a suicide.

In the first case, the scene and the injuries were analyzed for a woman found dead in her home. Death was caused by two different weapons: a knife and a stick. In this case, the dynamics, the phases of the event, and the simultaneous use of two weapons were not clear. In the second case, the death of a young boy killed at the door of his home by multiple gunshot wounds was assessed. In this case, the murderer declared a legitimate defense and the unwillingness to kill. Therefore, in this case, the use of this method was important to clarify the truthfulness of the declarations and the possible compatibility of the witnesses' stories. In the third case, a boy was evaluated who suffered a stabbing that caused cutting wounds in the pubic region. The aggressor declared his unwillingness to cause death but only the will to hurt the victim. The reconstruction with the application of the 3D model proposed by this study clarified any possible dynamics with analysis of the authenticity of the aggressor's statements. In the fourth case, the death of a man due to a firearm injury was analyzed. The analysis of the scene did not clarify the mode of death, as the body site affected was the left axillary region. Several dynamics compatible with the weapon used and the lesion analyzed were presented through the proposed experimental model.

In all cases, the dynamics were reconstructed using 3D MOCAP technology. The use of the method clarified the phases of the murder in the first case and assessed the mismatch of the aggressor's statements in the reasons for the crime of the second case. In the third case, it clarified the aggressor's intent to kill. In the fourth case, it clarified the suicide-type modality organized by the victim. In all cases, the reconstructions had a crucial role in court as tools to explain the hypothesized dynamics.

### Forensic Sciences, 3D Motion Capture, Autopsy



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### E84 Geolocating and Protecting the Grave Sites of the Disappeared in Lebanon

*Malena Gonzalez Eichenberg, BS, Act for the Disappeared, Beirut, LEBANON; Marwan Khoury\*, University of Leicester, Leicester, Leicestershire LE1 7RH, UNITED KINGDOM*

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**Learning Overview:** The goal of this presentation is to show how the Act for the Disappeared (ACT) started to document the fate of the missing of Lebanon, three decades after the end of the civil war. Attendees will be exposed to the methodology behind a preliminary open-source data gathering (newspapers, images, videos, official reports), the interviewing of direct sources (former fighters, witnesses, and survivors), and the crosschecking of different sources. This resulted in an investigative tool formed by a comprehensive database with interactive mapping that updates in real time as the data is modified. ACT will also share the necessary measures that it took to protect the sensitive findings, as well as a number of gravesites that were deemed at imminent risk.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by outlining how a local initiative led a serious citizen's-based investigation, after it became clear that successive governments were not prepared to undertake any meaningful action that would address the issue of the forcibly disappeared in Lebanon. ACT defied the odds that are defined by decades of post-conflict passivity resulting in data loss and grave site destruction and started collecting all remaining evidence. The methodology and the data management tools were developed with the support of the Argentine Forensic Anthropology Team (EAAF) and the International Committee of the Red Cross (ICRC), which in turn provided training on the scientific investigation of cases of political violence and the storing and archiving of sensitive confidential data.

ACT is a Lebanese human rights association founded in 2010 whose mission is to contribute to the clarification of the fate of the forcibly disappeared and missing of the Lebanese civil war. Approximately 17,415 persons went missing during this 15-year long conflict (1975–1990— as recognized by the Lebanese government. To this day, thousands of families are still waiting for information on the fate of their loved ones. In 2015, to complement the ICRC project of Ante-Disappearance Data (ADD) collection that started in 2012, and the Biological Reference Samples (BRS) collection that started in 2015, ACT started the collection of background information together with the mapping and the protection of grave sites. This work resulted in an investigative tool based on a comprehensive database with interactive mapping that updates in real time as the data is modified. The tool is built on Node.js and MongoDB following The Open Web Application Security Project (OWASP) security standards. The categories of the data include the missing person's case, checkpoints, detention centers, events related to cases of disappearances, potential grave sites, armed groups, contacts, and sources. The gravesites are ranked by three indices: risk of destruction, credibility of the information, and sensitivity of the site. Converging all these variables allows the filling of gaps and the association between individual cases and potential burial location and the location of the armed group's areas of control, checkpoints and detention centers, and links between previously unassociated cases of disappearances. Crosschecking such derived hypotheses with the ICRC's ADD and the postmortem examination results would increase the strength of the evidence and, therefore, facilitate identification. ACT also continues to work on the protection of potential gravesites to avoid their destruction.

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#### Missing Persons, GIS Mapping, Grave Sites





## E85 The National Institute of Standards and Technology/National Institute of Justice (NIST/NIJ) Evidence Management Initiative

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**Learning Overview:** After attending this presentation, attendees will learn about the activities of the NIST/NIJ Evidence Management Steering Committee (EMSC) including: the results of a national survey of evidence handlers, progress regarding the development of best practices regarding the retention and preservation of evidence types, and deliberations held at the Evidence Management Conference in October 2019.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that maintaining the integrity of physical evidence is a fundamental aspect of the administration of justice. Every day, critical decisions are made to determine outcomes in legal proceedings based on evidence and how it is managed. Once identified as evidence, the item begins a long and often complex journey until its final disposition (e.g., destruction, return to owner). Depending on the type of case, the type of evidence, the size of the item, and numerous other factors, an individual piece of evidence could be held for decades. Short- and long-term maintenance of the evidence involves a wide range of stakeholders, each with different organizational roles and responsibilities in the context of the larger system of justice.

In 2016, Congress passed the Justice for All Act calling for the “establishment of best practices for evidence retention” in consultation with federal, state, and local law enforcement agencies and federal laboratories. The NIJ commissioned the NIST to lead the development of evidence retention guidelines due to previous collaborations in the development of best practices for biological evidence published in April 2013. In March 2018, the EMSC met for the first time and developed a plan to complete its charge to: (1) develop best practices for the retention, preservation, integrity, and disposition of evidence and property; and (2) encourage adoption through education and engagement of the broad community of United States justice system stakeholders involved in the management of evidence.

Numerous studies and government-sponsored publications identify the need for best practices in the storage, preservation, and disposition of physical evidence. As the use of forensic evidence in criminal proceedings increases, the need to properly maintain the integrity of that evidence becomes pronounced. The national crises of untested sexual assault kits resulted in the passage of the SAFER Act of 2013 with revisions in subsequent years, which is symptomatic of a larger, systemic issue in the management of physical evidence. The NIST and NIJ 2013 report addressed storage issues in biological evidence, and the authors state: “Many law enforcement agencies do not recognize or support the efforts of their property rooms. Although these agencies bear ultimate responsibility for maintaining the integrity of the evidence, the real problem is a system-wide failure to properly account for evidence from collection through final disposition.”<sup>1</sup>

This presentation will include discussion of the activities that EMSC took to begin addressing the aforementioned issues. These activities include: (1) a national survey of evidence handlers and organization heads to learn more about existing gaps; (2) a review of the literature to support evidence preservation, storage, and disposition and identify gaps in the literature related to evidence handling and storage; (3) a public conference for federal, state, and local government representatives, communities of practice, and the public stakeholders to discuss, prioritize, and publicize evidence management issues and solutions; and (4) a report summarizing the findings of the previous three activities and identify best practices in evidence management in preservation, storage, and disposition.

This presentation will provide a summary of the findings of each of these activities and provide further opportunities for attendees to become engaged in the activities of EMSC.

### Reference(s):

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### Evidence Management, Preservation, Stability



### E86 Challenges in Establishing an Innocence Project in the Philippines: The Innocence Project Philippines Network (IPPN) Experience

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**Learning Overview:** After attending this presentation, attendees will better understand the background and history of the IPPN and the challenges it faced in organizing an Innocence Project in the Philippines.

**Impact on the Forensic Science Community:** The current Philippine administration has placed the re-imposition of the death penalty as punishment for drug trafficking and other drug-related offenses as one of its priority bills. This presentation will impact the forensic science community by exploring why an organization like the IPPN is needed now more than ever to ensure that no wrongfully convicted person is executed.

Inspired by the success of the first Innocence Project started by Peter Neufeld and Barry Scheck in 1992 at the Benjamin J. Cardozo School of Law, and the adoption by the Philippine Supreme Court in 2007 of the Rule on DNA Evidence, which provided for the first time a provision on post-conviction DNA testing, the IPPN was established in 2012 as a network of law school legal aid clinics, scientific and academic laboratories, and Non-Governmental Organizations (NGOs) that sought to make justice accessible to wrongfully convicted persons using DNA evidence.

IPPN has attracted volunteer lawyers and law and biology students from the following: (1) College of Law, Ateneo de Davao University; (2) College of Law, De La Salle University; (3) College of Law, University of Lipa; 4) College of Law, San Sebastian de Recoletos; 5) College of Law, College of Science and the DNA Analysis Laboratory, Natural Sciences Research Institute, University of the Philippines, and (4) Free Legal Assistance Group (FLAG).

The specific objectives of IPPN are: (1) to provide coordinated free legal assistance to persons wrongfully convicted; (2) to advocate reforms in policies, law, judicial procedures, criminal investigation procedures, and evidence handling; (3) to enhance capacities of justice stakeholders; and (3) to establish an independent and accurate databank on wrongful convictions in the Philippines.

After its launch in December 2012, IPPN conducted prison visits to the National Bilibid Prison and the Correctional Institute for Women where volunteer lawyers and law students interviewed prisoners who claimed to have been wrongfully convicted in order to find possible cases for post-conviction DNA testing. IPPN then conducted a Summer Internship Program in 2013 to train law student volunteers and is presently engaged in information awareness campaigns through social media and screenings of *Give Up Tomorrow*, a documentary involving a wrongful conviction case. In 2018 and 2019, IPPN conducted summer workshops with a prison visit to the minimum security compound of the National Penitentiary for volunteer interns to generate interest in the Innocence Project and to encourage the next generation of lawyers and health researchers to develop a passion for this type of advocacy.

One major challenge that IPPN discovered was the absence of properly collected and stored biological evidence in the cases where post-conviction DNA testing was being sought. Unlike other jurisdictions, there simply was no adequate and reliable system in place for the long-term preservation and collection of crime scene evidence. Thus, identifying and retrieving physical evidence for possible DNA testing proved impossible at this stage. In fact, in one celebrated case where DNA testing was ordered by the Philippine Supreme Court, no DNA test could be conducted since the biological evidence recovered from the crime scene could no longer be found several years later. A second challenge was the lack of a readily available sexual assault investigation kits so that biological evidence could be properly collected, preserved, and stored in cases of sexual assault within 72 hours post-contact. Another challenge is the absence of a law to create a forensic DNA database, which would have greatly assisted in the investigation of crimes. To date, IPPN has not yet found a single case in which the provision on post-conviction DNA testing could be applied to seek the release of a wrongfully convicted prisoner. Yet IPPN remains hopeful since the Philippine Supreme Court itself had earlier recognized and released statistics in the case of *People vs. Mateo* that showed a high judicial error rate of 71.77% in capital cases decided by the trial courts.<sup>1</sup> The need for forensic evidence such as DNA to close this gap becomes even more imperative.

#### Reference(s):

<sup>1</sup> *People vs. Mateo* (G.R. No. 147678-07, 07 July 2004).

#### Innocence Project, Wrongful Conviction, Forensic DNA Evidence



## E87 Terrorist Attack: The Identification of Destructive Lesions

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**Learning Overview:** The goal of this presentation is to demonstrate the methodological approach to wound evidence that comes from different weapons used on the victims of terrorism in war zones. The focus is on the main macroscopic elements used for the preliminary distinction between different weapon injuries in order to guide the initial phases of the investigations and for the identification of problems following destructive lesions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of the need to create a task force made up of forensic specialists, pathologists, radiologists, and dentists ready to intervene quickly in order to carry out the preliminary crime scene investigations, external examinations, and identification of the bodies in cases of death due to terrorist actions. The preventive acquisition of elements capable of allowing personal identification (fingerprints, radiographic assessments, and genetic typing) is necessary for military personnel engaged in high-risk areas.

In the past 20 years, terrorism, especially that linked to Islamic fundamentalism, has arisen all over the world and won the front pages of newspapers due to the brutality and the number of victims, including both soldiers engaged in peacekeeping missions and civilians in the West world as well as in the Middle East. A collection of cases of death by terrorist attacks will be presented.

Explosions, firearms, and air crashes are the main manner of killing. Explosions are the most frequent, causing almost 80% of the deaths, with 71% of deaths being military personnel, 22% being civilians, and 7% being hostages—both civilian and military.

In deaths due to explosions, the principal means used are cars or wagons filled with explosives, mixed with various types of metallic material added to increase the damage resulting from their projection in the space surrounding the explosion. The lesions found on the bodies of the victims vary according to the distance from the explosion: bodies found in the immediate proximity showed blast injuries with destructive lesions but also vast burns; the bodies found at greater distances showed missile injuries deriving from multiple splinters as minor as they were distant from the center; the bodies found farthest away mainly showed crushing injuries due to the collapse of buildings. The identification of the victims was often a major problem, especially for bodies found near the center of the explosions.

In deaths due to gunshot, the most used weapon is the gas-fired selective assault rifle Avtomat Kalashnikov (AK) both in the primal version (AK-47), firing the 7.62x39mm cartridge, and the next version (AKM-74), firing the 5.45x39mm cartridge. The macroscopic form of the entry wound as well as the exit wound was not regular and depended on the surface it traveled through before the impact with the body (e.g., car, doors) and during the intracorporeal trajectory (bones). Gunshots were also the usual cause of death among hostages. In these cases, the body is usually found several months after death, which often poses a problem related to the identification of the victims and to the establishment of the time of death.

Air crashes in the war zone principally refer to helicopters, which are the most-used means and, therefore, the most exposed to mechanical failures; and they are often targeted by enemy artillery or snipers. The external examination of the bodies recovered after a helicopter crash showed great precipitation trauma as well as heat lesions. In these cases, similar to explosion deaths, identification was not always possible due to the presence of destructive lesions.

During the initial phases of the investigations, the identification data of all the possible victims was preliminarily collected. These data had been crossed with the elements coming from the crime scene investigation (clothes, objects, identification plates) and the examination of the corpses (connotations, tattoos, dental prostheses). The collection of the circumstantial data, together with the external examination of the bodies with the macroscopic evaluation of the lesions, was often conclusive regarding the means of death in bodies with destructive injuries. Complete autopsies were then performed on the bodies and bone (femur) samples were taken for subsequent genetic investigations.

### Terrorist Attack, Destructive Lesions, Methodological Approach

## E88 Bovine Meat Fraud and Adulteration in Brazil: Innovative Techniques for Tamper Detection

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**Learning Overview:** After attending this presentation, attendees will understand important aspects about economically motivated food fraud in Brazil, specifically meat fraud processes, and new approaches using spectroscopic techniques combined with chemometric tools to detect illegal adulterations in bovine meat *in natura*.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing techniques with great potential for applications as a screening method, especially in regard to meat fraud identification.

World meat production has been growing at a rate of 0.64% per year. Alongside the United States, which accounted for 19.50% of total world production in 2018, Brazil is one of the world's largest meat producers (15.75% in the same year). The country accounted for 19.89% of all meat exported in the world last year, equivalent to 1.64 million tons (\$ 6.57 billion).<sup>1</sup> This corresponds to a growth of 7.9% over the previous year.

Due to the high demand for quality characteristics of fresh meat for export, concerns about the authenticity of food have been growing in recent years. In 2013 in Europe, one of the most famous incidents involving horsemeat tampering occurred.<sup>2</sup> In Brazil, the Federal Police had several scandals in recent years. In 2012, major food processors were investigated for bovine meat fraud by adding non-meat ingredients.<sup>3</sup> In 2017, the meat fraud in Brazil had the highest visibility and international impact with "Operation Weak Flesh."<sup>4</sup> The focus of this investigation was on the verification of irregularities, such as the use of animal carcasses for food production in other processing units, use of additives not permitted by law, bribes to federal inspectors and enforcement agents, and corruption.

The official methods for testing meat authenticity have employed classical techniques used for meat quality control, determining physico-chemical and microbiological parameters. However, these methods are time-consuming, laborious, and these individual parameters are inappropriate to characterize fraud due to the wide variation in the chemical composition of bovine meat, as a function of sex, cut, breed, feed intake, and slaughter age. On the other hand, the utilization of spectroscopic techniques combined with chemometric tools has shown great potential for forensic application as a screening method, because it provides fast, low-cost, minimally destructive analytical methods and requires little or no sample preparation.

In this work, the use of Raman hyperspectral imaging spectroscopy as a screening tool for fraud detection in bovine meat *in natura* by the addition of solutions of sodium salts and polysaccharides with the intention of increasing the water-holding capacity and, consequently, of obtaining economic gain. The mapped spectral range was from 2,400cm<sup>-1</sup> to 400cm<sup>-1</sup>. Data were processed using the HYPER-Tools graphical interface, and chemical maps were constructed using the Multivariate Curve Resolution (MCR) algorithm. As a result, the use of Raman imaging spectroscopy, combined with a chemometric tool, proved to be a promising method to help enforcement agencies fight against food fraud and adulterations. The use of these techniques facilitate this process, saving time and reducing costs.

### Reference(s):

1. OECD/FAO, OECD-FAO *Agricultural Outlook 2019-2028*. Paris: OECD Publishing and Rome: Food and Agriculture Organization of the United Nations. 2019. <http://www.fao.org/3/ca4076en/ca4076en.pdf>.
2. FSAI. FSAI survey finds horse DNA in some beef burger products. *Food Safety Authority of Ireland*, 2013 Jan 15. <https://www.fsai.ie/details.aspx?id=11878>.
3. Karen M., Nunes K.M., Marcus V.O., Antônio M.P., Filho S., Lasmar M.C., Sena M.M. Detection and characterization of frauds in bovine meat *in natura* by non-meat ingredient additions using data fusion of chemical parameters and ATR-FTIR spectroscopy. *Food Chemistry*. 2016;205:14-22. <https://doi.org/10.1016/j.foodchem.2016.02.158>.
4. Phillips D. Brazil meat scandal is called "a punch in the stomach." *The New York Times*. 2017 Mar 22. <https://www.nytimes.com/2017/03/22/world/americas/brazil-meat-industry-scandal-exports.html>.

### Meat Adulteration, Hyperspectral Images, Raman Spectroscopy



## E89 Multiple Implications of Postmortem Computed Tomography (PMCT) in the Forensic Approach to Charred Bodies

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**Learning Overview:** The goal of this study is to carry out an operating protocol that provides the radiologist with keys to establish complete and focused reports in all cases of PMCT of burn victims. The radiological investigation will be addressed according to the specific needs of the case in order to differentiate between normal postmortem changes from heat-related changes and to help the pathologist in different issues ranging from the victim's gender identification to localization, foreign bodies, or possible sites for fluid and DNA sampling.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by using the results obtained to optimize, validate, and promote the operative protocol used in such a way that it can be consistently applied in all cases of burned/charred bodies. According to the needs, the radiologist must discern all contextual divergences with forensic history, and must be able to report all relevant elements.

Although a fire generally does not completely destroy a body, in forensic pathology the correct examination of a burned/charred body is a challenge. Over recent years, imaging methods have gained ground in all fields of forensic pathology, where PMCT prior to postmortem has proven useful as an accessible and contemporary tool for forensic investigations. By using PMCT, important information supplementary to the traditional postmortem is obtained. In addition, PMCT provides insight into areas not routinely investigated during postmortem, such as the facial skeleton, basilar skull, cervical spine, arms, and legs. Furthermore, PMCT provides many others advantages, such as objectivity, repeatability, and 3D rendering of the body surface, allowing the pathologist to choose the dissecting technique most appropriate to the characteristics of the case, avoiding improper techniques that can led to damage of the examined structures or loss of important elements. An advanced state of carbonization often complicates surgical dissection and some foreign bodies (bullets, prostheses, etc.) or bone alterations (osteosynthesis, traumatic fractures, etc.) could be missed. PMCT provides specific semiologies requiring a prudent understanding to differentiate between normal postmortem changes from heat-related changes and can help the pathologist in the victim's identification process, guiding him in the autopsy, and in the localization of possible sites for collecting DNA or fluid samples. It is also important to perform histological examinations to highlight the presence of fat embolisms in the lungs as a sign of vitality at the time of burn.

The main objective is to use the results obtained to optimize, validate, and promote the operative protocol used in such a way that it can be consistently applied in all cases of burned/charred bodies. According to the needs, the radiologist must discern all contextual divergences with forensic history and must be able to report all relevant elements, in order to answer the following questions: Are there tomographic features that could help identify the victim? Personal Identification: Point out the presence of metallic objects stuck inside the body that are highly radio-opaque and thus easily seen on the PMCT (jewelry, watches, etc.). Personal Identification: Point out the presence of internal medical devices that are useful when reported in order to correlate with the medical record of the alleged victim (vascular prosthesis, osteosynthesis equipment, dental fillings, surgical clips, pacemaker, intra-uterine device, etc.). Gender Identification: In extreme cases in which the corpse is in such a damaged state that the victim's secondary sexual characteristics are no longer distinguishable and the gender can no longer be defined. Nevertheless, the deep organs are relatively preserved from the heat by the abdominal wall or peritoneum. Thus, the uterus or prostate are generally present on PMCT, allowing a determination of the subject's gender. Is there evidence of remains of biological fluids in liquid form available for toxicological analysis and DNA sampling? (The collection of fluid samples, during the autopsy, is particularly important in the case of a charred body. In addition to the usual toxicological screening, forensic personnel would seek to ascertain the percentage of serum carboxyhemoglobin in order to determine whether death occurred before or during the fire. The availability of biological fluids is inversely related to the burning level of the body and the main risk is that the few biological fluids present could be lost during the surgical dissection. A detailed imaging analysis of the PMCT can be extremely useful to point out possible collection sites). Is there another obvious cause of death other than heat-related lesions, especially metallic foreign bodies of ballistic origin? PMCT will show the presence of foreign bodies (bullets, knives, blades, etc.) or radiological findings different from typical heat-related lesions (bone fractures different from typical heat-related fractures, epidural collections with a subdural appearance, etc.) that are capable of causing traumatic death. What are the characteristic burn-related injuries seen on the corpse that should be sought during the autopsy?

### Postmortem Computed Tomography, Charred Bodies, Forensic Science



### E90 “Giant” Aneurysm of the Right Coronary Artery—The “Fortuitous Event” in Road Traffic: The Relevance of the Judicial Autopsy

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**Learning Overview:** A “fortuitous event” is seen as a circumstance precluding “wrongfulness” or providing exoneration from responsibility in wrongful acts. The goal of this presentation is to report on a unusual case in which the death of a cyclist victim of a road accident was not attributable to the injurious consequences of the collision with the vehicle involved, but to a thrombosed giant right coronary artery aneurysm leading to a sudden natural death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by underlining the importance of the judicial autopsy in the event of a traffic accident fatality in order to verify the existence of a causal relationship between the accident and the death, which can lead to the recognition, or the exclusion, of any liability.

A 73-year-old man riding his bicycle was involved in a car accident, being hit by a truck. Upon arrival of the ambulance, the man was declared dead. Since there were no witnesses, the dynamic of the accident was unclear. The external examination itself, disposed by the prosecutor, showed few abrasions and lacerated/contused wounds in the right parieto-occipital region and in the left parietal region of the head, not allowing the identification of the cause of the death. Therefore a judicial autopsy was performed, which disclosed a large, tumor-like, sacular-shaped aneurysm of the right coronary artery measuring approximately 3.5cm in diameter. The internal wall of the aneurysm was covered with old thrombotic material, and the remaining lumen was obliterated by freshly clotted blood. The heart appeared dilated and weighed 530g, characterized by degenerative valvulopathy. There was significant atherosclerosis in the aorta and the large arteries, but no other aneurysms were found. Examination of the remaining internal organs revealed no other significant pathological conditions. No skull fractures nor encephalic injuries were found. The aneurysm was embedded for histology, and paraffin sections were stained with hematoxylin and eosin. Histological examination confirmed the different ages of the layers of thrombotic material. The vessel wall of the aneurysm was thinned and hyalinized with focal calcifications. Based on the detailed postmortem examination and the histopathological study of tissues, the cause of death was attributed to a thrombosed giant right coronary artery aneurysm.

Aneurysmal Coronary Artery Disease (CAD) is an abnormal dilatation of a localized or diffuse segment of the coronary artery tree. The incidence of coronary aneurysm among CAD is about 1.5% to 5%. A coronary artery aneurysm with a diameter of >2cm, which is termed a “giant aneurysm,” is even more rare. It may be congenital, or secondary to other diseases, such as atherosclerosis. Coronary artery aneurysms may exist without symptoms until a fatal event occurs.

A more common complication is thrombosis in the area of the aneurysm resulting from an alteration of blood flow or a defective endothelial layer. In case of complete luminal obstruction or when parts of the thrombus are embolized in more distant branches of the coronary artery system, acute myocardial infarction is almost inevitable. Thrombosis of the aneurysm and consecutive myocardial infarction was also responsible for death in the present case, which is remarkable for several reasons.

Primarily, the importance of judicial autopsy in the forensic medical diagnosis of death following a traffic accident emerges, and postmortem examination should always be performed, especially when there is the involvement of elderly subjects. Moreover, in road accidents characterized by modest impairment or low-speed dynamics, it is always necessary to identify the cause of the death and whether the people involved in the accident had any diseases that might be the responsible for a sudden natural death instead of a traumatic one, leading to the solution of crucial medicolegal problems.

#### **Giant Aneurysm, Fortuitous Event, Judicial Autopsy**



### E91 The Abuse and Misuse of Prescription Opioids: Is It Only an American Problem?

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**Learning Overview:** The objective of this study is to analyze the Italian context to better understand if there is the possibility of an epidemic of addiction, as is the case in the United States and Canada.

**Impact on the Forensic Science Community:** This study will impact the forensic science community as many studies have analyzed the American and Canadian “epidemic of addiction” of prescription opioids, but very little has been written on the European situation, including Italy.

Medical use of prescription opioids has been constantly increasing since the 1990s, particularly in the United States and Canada, together with the misuse and abuse of these substances, and a significant rise in the rates of dependent individuals and deaths related to prescription opioids. The situation has become so dramatic, it has led the American authorities to speak of an “epidemic of addiction” and introduce a series of countermeasures in order to better face the problem. Even in Europe, there has been an increasing use of prescription opioids and related problems, but the European context is much less dramatic than the American and Canadian ones. Nevertheless, data does not allow the exclusion of the possibility that a similar crisis will also occur in the “old continent.”

Considerable concern surrounds tramadol, whose prescription is not placed under specific control as is the case for other opioids. The objective of this study is to analyze the Italian context to better understand if, as for the United States and Canada, there’s the possibility of an epidemic of addiction. A detailed review of autopsy records and toxicology reports was conducted at the Forensic Institute of “Sapienza” University of Roma to find cases of deaths related to the use of prescription opioids. The initial list of cases was compiled based on searches including the terms “overdose,” “opioid,” and “synthetic opioid.”

More than 170 cases were reviewed based on these search parameters. Only cases involving deaths related to the use of prescription opioids were included in the final dataset. After the vetting process, 60 different case numbers were found between 2014 and 2019 that pertained to deaths related to the use of prescription opioids. It was not possible to analyze the years prior to 2014 because prescription opioids were not routinely searched.

The cases of death, following the assumption of prescription opioids, were analyzed through the preparation of toxicological samples prepared with routine methods, followed by systematic screening for substances by diverse methods. Volatile compounds were identified using Gas Chromatography/Flame Ionization Detection (GC/FID). Medical drugs and drugs of abuse were identified via the use of Gas Chromatography/Mass Spectrometry (GC/MS) and liquid chromatography/tandem mass Spectrometry (LC/MS/MS).

Of a total of 60 subjects, 44 died due to an overdose of tramadol; while 12 died due to an overdose of buprenorphine, and 4 due to fentanyl overdose. Of the deceased, most were males and the most-used drug was tramadol. In all cases, the macroscopical examinations did not reveal any organ abnormality that could explain deaths. Through histological examinations with hematoxylin/eosin, no significant findings emerged apart from widespread edema and focal sclerosis of the myocardium, and interstitial and alveolar edema of the lungs.

This work shows that attention must be paid to prescription opioids, especially those considered “safer” as tramadol and buprenorphine. The fact that there is not a full-blown epidemic concerning the deaths related to the use of these drugs does not justify a carelessness of European institutions and they must implement preventive measures that avoid the recurrence of the North American situation.

#### Opioids, Dependence, Pain



## E92 On-Going Decision Analysis (a.k.a. Black Box) Studies at the Federal Bureau of Investigation (FBI) Laboratory

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**Learning Overview:** After attending this presentation, attendees will understand the development and purpose of the FBI's Footwear Examiner Decision Analysis Study, Handwriting Examiner Decision Analysis Study, and Firearms Decision Analysis Study, commonly known as "Black Box" studies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing the concerns of the results from the Decision Analysis Studies (DAS) presented in the 2009 National Academy of Sciences (NAS) Report on "Strengthening Forensic Science in the United States" and the 2016 President's Council of Advisors on Science and Technology (PCAST) Report on "Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods" with regard to narrowing gaps within "feature-comparison" methods of footwear, handwriting, and firearms disciplines.<sup>1,2</sup>

In early 2016, the Organization of Scientific Area Committees (OSAC) Footwear and Tire Subcommittee and Forensic Document Examination Subcommittee identified a major gap in current knowledge with regard to the reliability of Forensic Footwear Examiners (FFE) and Forensic Document Examiners (FDE). The need for firearms/toolmarks examiners to support subjective opinions with logical, scientifically based explanations as the basis for their identifications was reinforced in the 1991 case of *Ramirez v. State of Florida*. Along with the needs identified by NAS, OSAC, and the courts, the PCAST study was centered on the reliability of an examiner's conclusion that an evidentiary item was likely to have been derived from the same source as a known exemplar. To date, few studies have been conducted to establish objective measures of examiner accuracy in the forensic feature comparison disciplines. Decision analysis studies are one means to test the reliability and accuracy of these disciplines. A DAS only considers the evidence provided to examiners and the conclusions reached. It does not examine the decision-making process of examiners.

Test packets were created in each study to simulate casework and were assembled so that the combination of questioned and known samples would provide participants with challenging comparisons. Known (K) and Questioned (Q) handwriting exemplars, footwear impressions, and bullet and cartridge casings were collected and used to create each QKset. The studies were all double blinded and adhered to Institutional Review Board (IRB) protocols. For example, in the footwear DAS, each examiner received 100 QKsets to examine and then submit their conclusions within a custom graphical user interface. In addition, participants in the studies were required to have been actively employed in their field within a specified amount of time (e.g., 2 years for FDEs).

The objective of decision analysis studies of FDEs, FFEs, and Forensic Toolmark Examiners (FTE) is to determine the rate at which examiners provide the appropriate forensic conclusion and, in turn, to estimate the rate of errors (accuracy). The consistency of responses between examiners (reproducibility) and their ability to reach the same conclusion when provided with the same specimens at a later time (repeatability) will be measured. When testing is concluded, accuracy, reproducibility, and repeatability will be quantified through statistical analysis. Any inter-relationships and correlations among the various factors will be explored and analyzed. Areas of improvement that warrant further study will be identified, and it will be determined what range of conclusions are appropriate. This knowledge can be leveraged by implementing a re-evaluation of examiners' suitability measures, training programs, and/or quality control methodologies. Results will be published in peer-reviewed forensic journals. These studies were administered in partnership with Ideal Innovations, Inc. and Noblis (FDE, FFE), and AMES Laboratories (FTE).

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### Feature Comparison, Decision Analysis, Black Box



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### E93 The Role of Postmortem Computed Tomography (PMCT) and Immunohistochemical Techniques in a Case of Aspiration Pneumonia in Suspected Sudden Infant Death Syndrome (SIDS)

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**Learning Overview:** The goal of this study is to analyze the way in which the combined use of instrumental and immunohistochemical methods allows a correct diagnosis of aspiration pneumonia in a case of suspected SIDS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how, in SIDS cases, autopsy findings can be non-specific. The PMCT can direct the forensic pathologist toward a certain etiology. In cases of asphyxia by aspiration, immunohistochemistry is an essential technique to clarify the nature of the aspirated material, as well as its presence within inflammatory cells. The combination of these two techniques can provide a valid diagnostic aid in complex cases, such as SIDS.

SIDS is defined as the sudden and unexpected death of an infant without any apparent cause. It has major prevalence in the male sex. Etiology of SIDS is still unknown. According to numerous authors, SIDS may be due to different causes, as neurological, endocrine, metabolic, pulmonary, immune, or cardiac, and 30%–40% of cases are due to pulmonary disorders and the aspiration of gastric contents is recognized as a cause, with the consequent development of aspiration pneumonia.

Aspiration pneumonia is an inflammatory process that develops from the entry of foreign materials into the bronchial tree, often coming from the oral or gastric route, such as food or saliva. Risk factors for aspiration pneumonia include all those pathological conditions that alter the state of consciousness or create dysfunction of swallowing. Usually, aspiration pneumonia is manifested by the sudden appearance of respiratory difficulties and cough, associated with the ingestion of solid or liquid food or the regurgitation of gastric contents. Regarding SIDS, the use of PMCT in cases of sudden neonatal death is explained by the fact that SIDS never presents a certain cause of death nor possesses characteristic findings in autopsy; moreover, CT is a useful method of screening for unnatural death.

In the present case, a 2-day-old male baby (born of a diabetic mother) was pronounced dead after suffering an acute lung failure and a cardiopulmonary arrest. The previous day, the baby was in a state of hypotonia due to persistent hypoglycemia and he experienced an episode of vomiting. A total body PMCT was performed. Pulmonary scan revealed the presence of hypodense material of indeterminate nature in the main airways, from the oropharynx to the subsegmental bronchi of both lungs, with areas of pulmonary parenchyma with a “ground-glass” pattern. The remaining sectors explored appeared to be free from alterations. At autopsy, the respiratory tract revealed the presence of an edematous mucosa, which was painted with a whitish filamentous material.

Histology samples of lungs were taken and processed in Hematoxylin-Eosin (H&E) staining. A pulmonary histological study showed strong congestion of the alveolar septa capillaries, which were stacked by white blood cells. There was a widespread reduction of airspace due to collapse of the lung texture. Macrophages with cytoplasm occupied by abundant amorphous material were visualized. The same amorphous material was found in the alveolar spaces and in the bronchi, together with a great amount of white blood cells. Furthermore, an immunohistochemical study was carried out on lung samples, using antibody reaction with CD15, CD68, and alpha-lacto-globulin antibody. The study with CD15 (neutrophil cell marker) and CD68 (macrophage marker) showed clear positivity in the lung parenchyma and alveolar cavities. This finding is decisive for an inflammatory pulmonary process prior to the death, confirmed by immunohistochemical tests.

Finally, this study proceeded to the immunohistochemical research of alpha-lactoglobulin (milk protein). In this case, alpha-lactoglobulin antibody reaction showed a marked positivity in the alveolar cavity site but particularly in the macrophage cytoplasm. This latter finding attributed the development of pneumonia to an inhalation of milk.

In SIDS, autopsy findings can be non-specific. The PMCT can direct the forensic pathologist toward a certain etiology. In cases of asphyxia by aspiration, immunohistochemistry is an essential technique to clarify the nature of the aspirated material, as well as its presence within inflammatory cells. The combination of these two techniques can provide a valid diagnostic aid in complex cases, such as SIDS.

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**SIDS, Aspiration Pneumonia, PMCT**



### E94 Epidural Hematoma in a Whiplash Cervical Injury: A Rare Condition

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**Learning Overview:** After attending this presentation, attendees will better understand the dynamics of whiplash with the possible deadly consequences it can determine.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how uncommon the following type of case is and how to deal with the consequences.

The name 'Whiplash Injury' (WI) derives from the etiopathogenic description of the sharp whipping movement of the head and neck, produced at the moment of a traffic accident, particularly following rear-end, head-on, or side collisions. The use of safety belts has led to a reduction in deaths derived from front-end collisions, but also to an increase in cases of typical whiplash. There are two types of force that cause WIs in rear-end car crashes: external forces applied to the body by the seat and head restraint, and internal forces generated by the activation of the body's muscles. The combination of these forces causes a differential motion between the superior and inferior tracts of the cervical spine (hyperextension-hyperflexion), which results in a strain of the neck tissues. This event can have consequences for all the neck structures: facet joints capsule, muscles, intervertebral disks, nerves, and vessels.

WI is characterized by a collection of symptoms that occur following damage to the neck, usually as a result of a sudden strain affecting the discs, muscles, nerves, or tendons of the neck caused by a sudden acceleration or deceleration of the head and neck. The head is violently thrown back, forward, or sideways, followed by reflex contraction in the opposite direction.

A condition that sometimes occurs after motor vehicle accidents are epidural hematomas. Although the pathogenesis of epidural hematomas remains widely controversial, there is a general consensus that all patients experienced the same mechanism of trauma since they were involved in a high-speed motor vehicle accident as a passenger, a pedestrian, or a bicyclist. Any deceleration, such as that occurring during cervical hyperflexion or hyperextension injuries, may result in a vascular lesion, with or without vertebral fracture

This study reports a case of a 68-year-old woman. The subject, while driving his car, hit another car head-on from the opposite direction and the woman in the hit car died immediately. During autopsy, there were no external signs consistent with fatal injuries. The only identifiable lesions were bruising in various body areas. The internal examination revealed no lesions of the head and brain; also, the organs of the abdomen had no particular alterations. There were multiple rib fractures with pulmonary laceration in the chest. When the neck organs were removed, there was evidence of infiltration of the perivertebral muscles. At the observation of the cervical vertebrae, there was a fracture of the fifth cervical vertebra. Diffuse epidural hemorrhage was also identifiable. Small hemorrhages were also found in the spinal cord.

Histological investigation confirmed the findings of the macroscopic examination. Surely the death of the subject was caused by the damage to the spinal column and spinal cord caused by the kinetic energy that developed due to a sudden acceleration followed by a deceleration. This dynamic caused vertebral fracture, epidural hemorrhage and bone marrow hemorrhage.

#### **Whiplash, Epidural Bleeding, Traumatic Injury**



### E95 Auxillary Artery Injury: A Rare Case of Death by Severe Blood Loss Due to an Accidental Fall

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**Learning Overview:** The goal of this presentation is to present a rare case of accidental sharp force death, showing how a thorough forensic pathology methodology, including death scene investigation, autopsy examination, and toxicological analyses, are pivotal to detect the manner of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community because of the rarity of this manner of death, regarding the epidemiological data about accidental death due to sharp forces.

Forensic pathologists are often asked to investigate deceased victims of stab wounds, as sharp force injury accounts for 10%–20% of clinical forensic examinations, and sharp force trauma represents the second most common cause of injury investigated in forensic practice. Moreover, homicide by sharp force (stabbing) is one of the most common causes of death in European countries, involving mostly domestic or interpersonal conflict. Stabbing as a suicide method constitutes a low percentage of all suicides, 2% to 3%. Accidental death due to sharp force is even rarer (0%–3%) and is usually caused by an impact or a fall into a different type of glass surface, with the victim generally being hit by a sharp-edged shard of glass, striking body parts with enough force to cause death. Death due to stabbing is usually caused by exsanguinating incisions to organs or large blood vessels, leading to hemorrhagic shock.

This study presents an unusual case of a man who was found dead at home, lying on a chair in a pool of blood with hundreds of glass fragments close by. During crime scene investigation, external examination of the body revealed a tear in the clothing at the right armpit. After removing the T-shirt, a 9cm-long wound was observed perfectly consistent with the tear. No other lesion was identified at the external examination during judiciary inspection. Analysis of the crime scene identified a shattered vase as the source of the glass fragments responsible for the armpit lesion.

Full autopsy was performed. At the external examination, the armpit wound was located 29cm away from the suprasternal notch and 40cm away from the xiphoid process; the wound presented clean-cut edges and the medial end presented a fishtail-like split. It was 3cm deep, involving pectoral muscles and surrounding fat tissue.

The internal examination did not show any gross alterations of organs. Dissection of the right arm musculocutaneous layers were performed showing hemorrhagic infiltration of the muscles, fat, and neurovascular bundle. On the posterior-lateral wall of the distal axillary artery, a 0.8cm linear full-thickness lesion was found. Histological study of the axillary artery confirmed the macroscopical finding. Further, toxicological analyses on blood, urine, and gastric fluid samples showed an alcohol concentration exceeding the toxic level, respectively 2.56 g/L, 3.55 g/L, and 2.48 g/L. Death was due to massive blood loss resulting from a lesion to the right axillary artery by glass fragments. A thorough analysis of scientific literature on death by sharp force injuries, due to axillary artery lesion, showed no results.

#### **Sharp Force, Axillary Artery Injuries, Accidental Death**

## E96 The “Social Web” Autopsy as an Evolution of Psychological Autopsy: The Application of the Method in Forensic Cases in Comparison

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**Learning Overview:** After attending this presentation, attendees will understand the evolution of the method of psychological autopsy in forensic cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the role of the web community in deaths from murder, suicide, and accident.

Psychological autopsy is a survey commonly used to study suicide deaths through the analysis of the family and social setting with the use of data collection through interviews with family members and psychological analysis of dynamics preceding death. However, to date its application has found an evolution in the era of globalization and for the diffuse presence of the web in the lives of individuals, so this study proposes its application to any forensic case whose dynamics are dubious.<sup>1,2</sup> In particular, the flow of information through telephone messages and chat and social networks has increased the means of social and environmental influence. Therefore, a web analysis can provide useful data for investigators, which may be unknown even to family members. A search of social networks is very important to investigate the victim’s psychology through the analysis of posts, images, and videos published in the weeks leading up to death. Together with the Personal Computer (PC), the mobile phone is an essential tool for the psychological autopsy.<sup>3,4</sup>

Reported here are three cases in which an evolution of the method of psychological autopsy was used. The analysis of the environmental, family, and social setting was compared with the evaluation of telephone, informatics, and social profiles of the victims. Furthermore, the data collected were compared with interviews of family members and acquaintances. The first case concerns a girl who was found dead in her home’s garden due to fall from height by suicide. The analysis of the house, in particular the girl’s room, highlighted the presence of drawings depicting stylized “souls” and other drawing proofs. Moreover, from the previously seized mobile phone, daily chats were found with obsessive virtual friendships and manipulative control not known by family members. In the second case, a boy was found dead due to an accidental railway accident. The analysis of the area allowed investigators to understand the dynamics of the event due to the use of the mobile phone to carry out a dangerous selfie with other friends who escaped. The cell phone was found two meters away from the body and was handed over to the police who subsequently analyzed the photos taken by the boy shortly before the accident. Family members did not know about the dangerous selfies, as opposed to schoolmates who did.

In the third case, researchers analyzed a double murder of a couple by firearm by the woman’s ex-boyfriend. The analysis of the social media profiles of the couple highlighted the presence of photos published on social media the day before the murder. The interrogation of the murderer revealed the motive of the crime, preceded by a story of stalking, after viewing the photos on social networks. The use of this method made it possible to answer some questions that clarified the manner, the motive, and the causes of death in all cases. Considering should be given to how much the environment can also influence the genesis of fatal events. Certainly in adolescents, the social-web is crucial in the genesis of accidental and suicidal events, as well as in the adult age group in the context of homicides, especially those with a passionate background. Currently, it is known how important it is to appear and be accepted, not only in everyday life but also on social networks, which often constitute the representation of a false self. This happens most in the adolescent age group where young people are constantly looking for a model to follow that allows them to make an idyllic escape in a parallel world.

This study proposes a method that represents a valid support to better analyze the setting, the manner (emulation), and the causes of similar events. It can be used both for investigative purposes in cases of homicide, suicide, or accidental deaths as well as for preventive purposes in the presence of explicit threats to things or people. During the investigation, it is useful to apply a multi-method approach to compensate for the limits of each and to have a broader view of the facts, thus facilitating the survey and prevention of announced fatal events.

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### Forensic Science, Psychological Autopsy, Social Web Autopsy

## E97 The Role of Social Networks in the Crime of Stalking: A Case of Murder

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**Learning Overview:** After attending this presentation, attendees will understand the role of social networks in investigating the crime of stalking.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the importance of applying the method of “Social-Mobile Autopsy” to prevent the crime of stalking before the stalker adopts violent behavior.

Social networks are digital platforms developed to allow communication between people who could be a great distance apart. These programs are known to also offer personal data sharing. From this point of view, social networks have become easy tools for acquiring private information via videos, geographic location, and fast methods of contacting other people. In this context, social networks may also be dangerous when are used improperly to achieve personal persecutions. The term “stalking” denotes a disturbing, threatening, unpleasant, repetitive, and/or intrusive behavior directed toward a specific person.<sup>1</sup> The victim is often linked by previous romantic relationships with the stalker, who does not accept the separation and begins an activity of controlling the victim with obsessive methods. Sometimes, the victim can be a person not known directly to the stalker but contacted through other methods, such as virtual dating sites.

A case is presented of double homicide by firearm that took place in a restaurant in a southern Italian town. The victims were a man and a woman who worked in the restaurant. Initially, the investigators had difficulty reconstructing the motive of the murder and the reason why the killer had chosen to kill only the two subjects among all the people present in the room. A crime scene investigation and an autopsy were carried out. Later, investigators listened to family members, who assumed an economic motive. Subsequently, the mobile phones of the victims and the killer and their respective personal computers were seized. The investigators focused the investigations on the social profiles of the three individuals using the Social-Mobile Autopsy method by analyzing messages, chats, public and private posts, photographs, and videos.<sup>2</sup> These investigations were carried out to understand the relationship between the victims with the killer and the motive for the murder.

The investigation revealed that the killer had used two firearms at the same time (a rifle and a gun). The woman had been struck first with both weapons for a total of 23 shots. Subsequently, the killer pursued the man on the street outside the room by hitting him only with the gun for a total of seven shots.

The analysis of the victims’ mobile phones revealed the exchange of messages between the two individuals and the existence of a hidden romantic relationship. However, the analysis of personal telephones did not highlight messages with the killer. Only the investigation of the victims’ social profiles revealed an intense stalking activity by the killer against the woman. The man sent numerous obsessive messages, photos of the victim, inserted numerous likes and comments in posts, and made video call attempts, often using fake accounts or other friends’ profiles. The murderer’s profile revealed repeated searches by typing his name several times in the search engine and publication of several posts related to the woman. The messages and photos suggested the woman’s previous relationship with the killer, who did not accept the end of their relationship.

Social networks have a fundamental role to get in touch and acquire information on the private lives of other people. In recent years, the so-called “stalker” has found in digital media the ideal tool to acquire personal information about the victim or to carry out the pursuit and oppression of the victim, specifically by sending hundreds of text messages, e-mails, and calls forwarded often using fake accounts. In the case described, the investigation carried out using the Social-Mobile Autopsy method allowed the discovery of a real crime implemented through social networks, such as stalking, and therefore allowed clarification of the motive for the murder. This case demonstrates the importance of using the Social-Mobile Autopsy method in investigating cases of suspected stalking in order to take preventive measures to ensure the safety of the victim before the stalker adopts violent behavior. Finally, this type of investigation is suggested in all cases of homicide/suicide in which the motive appears doubtful or cannot be reconstructed through circumstantial data or testimonies.

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### Forensic Sciences, Stalking, Social Networks



## E98 Optimizing Sensitivity and Validating the Illumina® Infinium Assay for Genotyping of Forensically Relevant Sample Types for Investigative Lead Generation

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**Learning Overview:** The goal of this presentation is to demonstrate a validated forensic workflow using the Illumina® Infinium assay capable of producing accurate Single Nucleotide Polymorphism (SNP) genotyping data for investigative lead generation with a higher sensitivity to lower DNA amounts and geared toward forensic-centric sample types.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by pioneering a standard validation for a forensic workflow for genome-wide SNP genotyping. Additionally, this presentation highlights the capabilities of this assay to be more sensitive, overcoming the 200ng standard input and essentially shifting the applicability of this technology from clinical laboratories to the forensic community.

Forensic genealogy applies enhanced genetic processing techniques (array-based genome-wide SNP genotyping) combined with traditional genealogical research techniques to produce new leads in cases that have gone cold or where traditional investigative means have been exhausted. The use of this technology in investigative forensics has skyrocketed since the 2018 arrest of Joseph DeAngelo as the Golden State Killer. Most microarray-based genome-wide SNP genotyping takes place under clinical research, providing services that are not adapted to forensically relevant sample types. Furthermore, Direct To Consumer (DTC) laboratory tests require high quality and quantity DNA. The Infinium assay workflow is a genome-wide microarray genotyping assay that utilizes the BeadChip platform.<sup>1</sup> This accurate and flexible microarray technology allows for the ability to interrogate a large number of SNPs through unlimited loci multiplexing.<sup>2-4</sup> However, overcoming the 200ng standard input for this assay is essential for forensic genomics as it is rare to obtain DNA at such high quantities from forensic samples.

Here is described a validation of Illumina's® Infinium assay using the Infinium Global Screening Array to show successful optimization and validation down to DNA input levels more consistent with forensic sample types and shifting the applicability from clinical laboratories to the forensic community. In an effort to set a standard for validating a forensic workflow to generate genome-wide SNP genotyping data, the validation design, where applicable, is guided by the current Federal Bureau of Investigation (FBI) Quality Assurance Standards (QAS) for DNA testing laboratories and the Scientific Working Group on DNA Analysis Methods (SWGDM) Validation Guidelines for DNA Analysis Methods.<sup>5,6</sup>

Precision and accuracy of the assay will be evaluated using the National Institute of Standards and Technology (NIST) standard reference material that have been extensively characterized.<sup>7</sup> The sensitivity study will be carried out using blood, buccal/saliva, and semen samples. These common sample types will be extracted, quantified, and diluted in a series ranging from the manufacturer-recommended input target of 200ng down to 1ng of total DNA input—an input amount more consistent to forensic samples. Currently, there are no computational methods to parse mixtures to individual genotypes. Given that mixtures are a possibility, a mixture study will be conducted to observe how data are presented at different mixture ratios. Identifying mixtures is also critical when assessing for possible contamination. Reagent blanks will be incorporated at extraction and carried throughout the workflow. Additionally, during the precision and accuracy study, the plate layout will be designed in a way to identify possible contamination during the assay. This validation will demonstrate a forensic workflow using the Illumina® Infinium assay capable of producing accurate SNP genotyping data for investigative lead generation with a higher sensitivity to lower DNA amounts and geared toward forensic-centric sample types.

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### Microarray, SNP Genotyping, Investigative Lead Generation



## E99 Suicidal Cut-Throat Wounds: Elements of Differentiation From Homicidal Slaughtering

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**Learning Overview:** After attending this presentation, attendees will know more about the crucial features in forensic evaluation of cut-throat wounds for differentiation between suicidal and homicidal deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a complete evaluation of the crime scene investigation and the external findings of a suicidal death, underlying the distinct elements that can represent conclusive data toward the suicidal or homicidal hypothesis.

Suicide by stabbing one's own throat remains very rare and very few cases have been reported in the forensic literature. Crime scenes can often be misleading, and the direction of stab wounds, their site and number, in addition to the presence or absence of defensive wounds and hesitation marks have particular importance in helping to differentiate suicidal from homicidal deaths.<sup>1,2</sup> This study reports on the case of a 75-year-old man, found dead by his wife, with a blood-smear kitchen knife in his hand. The man had a history of psychiatric disease (mixed anxiety-depressive disorder); he was on pharmacological treatment and supervised by a psychiatrist. The morning of the discovery, he had locked himself in the bathroom and after some time, his wife, having knocked at the door several times without receiving an answer, opened it with a second key and found him lying in a prone position.

External examination of the body was performed. Findings showed an irregular transverse 14cm-long throat cut. In detail, the lesion started from the right sternocleidomastoidal region (2.5cm beneath the lower jaw angle), involving the lower part of the upper hyoidal region and the supero-medial part of the lower hyoidal region, ending at the left sternocleidomastoidal region. The underlying anatomical structures were exposed (uvula, back wall of the hypopharynx, and epiglottis). The carotid commune arteries weren't damaged. The edges of the wound were abraded and ecchymotic, and the upper part presented two epidermal flaps in its context. The right tailing was 2.5cm long and, above and below it, there were several linear, parallel cuts (three above and two below), the longest being 3cm. Another linear cut, 10cm long, 1cm above the right end, approximately parallel to the lower jaw, and four other linear abrasions, starting from the right medial part of the upper edge and going slantwise with below-upward lateral-medial and almost parallel direction, with a maximum length of 3cm, were detected. Last, another linear transversal cut of 2.5cm, starting from the upper edge of the left epidermal flap, was found. These lesions were recognized as the typical, self-inflicted suicide hesitation marks (superficial, parallel, sharp-forced skin cuts).<sup>3</sup> No significant findings were revealed on the arms, hands, back, or other parts of the body, leading to the conclusion of the absence of defensive wounds. A farewell letter was not found on the scene.

The presence of evident tentative wounds, the absence of defensive lesions, the medical history of the deceased, and the circumstantial and crime scene elements led to the conclusion of suicide. This case highlights the specific features that can help the pathologist discriminate between accident, homicide, and suicide. The complete evaluation of the crime scene, including the eventual presence of a farewell note or of elements of disturbance, and of the external findings on the body (defensive wounds, hesitation marks, lesions' typology and features) can significantly help the pathologist in correctly establishing the manner of death.

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### Forensic Pathology, Suicidal Cut Throat Wounds, Slaughtering



## E100 Forensic Science in the United States Court of Appeals: A Ten-Year Review (2009–2018)

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**Learning Overview:** After attending this presentation, attendees will understand: (1) which scientific disciplines are most frequently associated with appeals relating to the introduction or exclusion of scientific evidence; (2) which forensic science disciplines are challenged; and (3) how legal issues for appeal vary by scientific discipline.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing the forensic science disciplines that are appealed in the United States Courts of Appeals. In recent years, there have been efforts to look at the underlying validity and reliability of forensic science evidence from criminal and civil law and regulatory issues.<sup>1</sup> While there have been some efforts to look at legal challenges to scientific evidence, specifically surrounding the impact of the United States Supreme Court's 1993 *Daubert v. Merrell Dow Pharmaceuticals* decision, an updated effort to catalog the diversity of scientific domains that are called upon in the course of criminal law, and in what contexts, would be beneficial.<sup>2-4</sup> For instance, identifying the scientific disciplines that are most frequently associated with appeals relationship to the introduction or exclusion of scientific evidence, how do legal issues vary by scientific discipline, and are there trends or areas of law as applied to scientific disciplines that require further attention from criminal justice stakeholders, including the forensic science community, courts, and trial attorneys.

The United States Courts of Appeals records were surveyed from the past decade (2009–2018) and categorized by scientific disciplines brought up on appeal. Lexis Advance was used to identify cases, using search terms that were adapted from previous literature.<sup>2</sup> The main goal of the search was to identify cases involving admissibility, Rule 702, and Rule 703 (rules addressing expert evidence in the Federal Rules of Evidence). The search terms were designed to be overly inclusive in an effort not to miss any cases in which the opinion rendered included expert evidence. The final search included 1,775 cases. Stratified random sampling, based on the circuit size, was used to identify a sample of 350 cases.

For the 350 cases identified, case opinions were reviewed based on a 28-question codebook pertaining to information about the case (e.g., report number, circuit, data of decision, and subsequent actions); items pertaining to the basis of appeal and secondary basis of appeal; and items pertaining to the evidence type, primary scientific discipline, and a broad categorization of discipline. If there was forensic testimony, there were items related to the broad category of forensic evidence (e.g., biology, digital/multimedia) and the expert education level (e.g., PhD, MD, DDS). The codebook was reviewed individually by two attorneys and two individuals in the forensic science community. Cases were excluded if the case was a civil case that was not a writ of habeas relief, the opinion contained insufficient detail to determine the background and type of expert witness testifying (i.e., no reference as to the expert witness' profession or domain of expertise), if the appeal was about lay opinion testimony, appeals focused on a search warrant, or the result of a false search hit.

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### Evidence, Appeals, Case Review





## E101 Time Between Sexual Assault and Evidence Collection: Implications for the Development of Combined DNA Index System (CODIS) -Eligible DNA Profiles

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**Learning Overview:** After attending this presentation, attendees will understand new research findings supporting that although the length of time between sexual assault and evidence collection can be five to six days post-assault, a CODIS-eligible Short Tandem Repeat (STR) DNA profile can still be developed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing victims, law enforcement, health care providers, forensic scientists, and the public with increased knowledge about the length of time in which victims can receive a Sexual Assault Forensic Examination (SAFE) to obtain an STR DNA profile of their perpetrator.

The methodology of the study is an exploratory, retrospective design of over 2,700 submitted and analyzed sexual assault kits from a Mountain West state in the United States. Findings will be compared to other research on time between sexual assault and evidence collection in the development of CODIS-eligible STR DNA profiles. Review of the current literature on time between assault and evidence collection indicate male DNA (Y-STR DNA) can be recovered up to seven days post-coitus from a cervico-vaginal swab and a Y-STR profile can be developed at eight loci.<sup>1</sup> In another study evaluating sexual assault cases, researchers found the longest time between the sexual assault and evidence collection that was positive for sperm was 169 hours (seven days).<sup>2</sup> Additional research supports possibly extending beyond the seven-day interval for DNA findings. Ballantyne and Speck discovered Y-STR DNA ten days post-coitus in a monogamous couple, demonstrating the longevity of male DNA in the cervix and vagina.<sup>3</sup>

In this large-scale, retrospective study of 2,727 sexual assault kits, probative STR DNA CODIS-eligible profiles were developed in 39% of analyzed kits. Generalized Estimating Equation (GEE) logistic regression analysis found that for every 24 hours that passed between assault and sexual assault forensic examination, there was a 10% reduction in the development of a CODIS-eligible DNA profile. The longest length of time between assault and forensic examination and development of a CODIS-eligible profile in the study was 122.5 hours, over five days from vaginal and cervical swabs. This finding supports the testing of DNA samples collected five to six days post-sexual assault due to the possibility of developing a CODIS-eligible profile.

This retrospective study is impactful due to the large sample size. The 2,727 sexual assault kits in this study are representative of sexual assault kits and findings in practice. The advancements of DNA testing paired with the research findings acquired from this study disprove the myth associated with delayed reporting and aid in educating victims, health care providers, law enforcement, forensic scientists and the public to extend evidence collection to over five days. In this study, full STR DNA probative profiles were developed from vaginal and cervical swabs obtained 122.5 hours after sexual assault.

### Reference(s):

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2. Suttipapit P., Wongwittayapanich S. Detection of prostate specific antigen and semenogelin in specimens from female rape victims. *J Forensic Legal Med.* 2008;54:102–8. <https://doi.org/10.1016/j.jflm.2017.12.017>.
3. Speck P., Ballantyne J. Post-coital DNA recovery study. *National Criminal Justice Reference Service.* Aug 2004. xiii-xiv. <https://www.ncjrs.gov>.

### DNA, Sexual Assault, Evidence Collection



### E102 Does It Wash Away? The Impact of Bathing or Showering on DNA Analysis Findings From Sexual Assault Kits

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**Learning Overview:** After attending this presentation, attendees will understand the minimal effect of post-assault bathing or showering on the development of the Federal Bureau of Investigation (FBI) Combined DNA Index System (CODIS) -eligible DNA profiles from Sexual Assault Kit (SAK) evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by encouraging the submission and analysis of SAKs despite a victim history of post-assault bathing or showering.

SAK submission rates significantly decrease in cases where the victim has engaged in post-assault actions, such as bathing or showering. The belief that these actions diminish the possibility of finding prosecuting evidence heavily contributes to this decline.<sup>1</sup> This belief must now be reevaluated in light of improved DNA analysis methods that can yield CODIS-eligible DNA profiles. The post-assault action of bathing or showering has been found to be a significant negative predictor of SAK submissions by law enforcement.<sup>2</sup> However, DNA can be collected from skin and successfully analyzed regardless of victim bathing or showering.<sup>3</sup> In an effort to eradicate misbelief and improve SAK submission rates, this presentation will evaluate SAK submission rates and SAK DNA analysis findings from 5,423 SAKs and provide research findings on the effects of post-assault bathing or showering on the development of probative CODIS-eligible DNA profiles.

In this retrospective study of 5,423 SAKs, 36% of victims reported post-assault bathing or showering. Post-assault bathing or showering was found to be highly correlated with time between assault and sexual assault forensic examination. In a Generalized Estimating Equation (GEE) logistic regression analysis on SAK submission rates, victim reports of post-assault bathing or showering was a consistent predictor of law enforcement not submitting kits. Yet, bivariable statistical analysis determined that victim bathing or showering post-assault was not associated with the lack of development of a DNA probative profile from SAK evidence.

The effect of post-assault bathing or showering must be reconsidered in the forensic science and criminal justice community as DNA analysis can yield probative DNA profiles, even after a victim has bathed or showered. The presentation of these research findings will encourage the submission of SAKs by law enforcement and potentially increase SAK submission rates by eradicating any doubt regarding the minimal effect of bathing or showering on the development of CODIS-eligible DNA profiles. At the conclusion of this presentation, attendees will understand the research data that supports the submission and testing of all SAKs, especially those with a victim history of post-assault bathing or showering.

#### Reference(s):

1. Patterson D., Campbell R. (2012). The problem of untested sexual assault kits. *J Interpersonal Violence*. 2012;27(11):2259-2275. doi:10.1177/0886260511432155
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#### Bathing/Showering, DNA, Sexual Assault Kit



### E103 Testing Sexual Assault Kits Leads to Justice for Both Victims and Suspects

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**Learning Overview:** After attending this presentation, attendees will have increased knowledge and competency on the importance of testing sexual assault kits to establish justice for both victims and suspects.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing research data supporting the mandatory submission of sexual assault kits to promote justice for all. Additionally, these findings can be shared with criminal justice colleagues to promote submission and testing of sexual assault kits and collection of suspects' DNA samples

In the United States, there is a push to mandate submission and testing of all sexual assault kits. A typically overlooked benefit of testing sexual assault kits is the exoneration of wrongfully accused suspects.<sup>1</sup> Sexual assault kits include DNA samples from the victim of sexual assault and occasionally are submitted with DNA samples collected from the identified suspect for comparison. Suspect samples may be taken by law enforcement personal or medical professionals in accordance with a court order.<sup>2</sup> Inclusion of suspect samples is beneficial because it can lead to a DNA match with samples collected from the victim or exclude the suspect as the source of DNA. The study methodology as an exploratory, retrospective design of submitted sexual assault kits with suspect DNA evidence samples was used to evaluate for the exclusion of suspects based upon DNA analysis findings. For a sexual assault kit to meet the criteria for "excluded the suspect," a DNA profile had to be developed from analysis of the sexual assault kit evidence that excluded the named suspect with the submitted DNA sample (either buccal swab or blood sample).

A large retrospective study of 2,727 sexual assault kits with completed DNA analysis found 66 cases in which the DNA findings excluded the suspect. Findings will be presented on descriptive data on these cases in which the named suspect was excluded: relationship between victim and suspect, victim loss of consciousness/awareness at time of assault, alcohol/drug use during the assault, suspected drug-facilitated sexual assault, and multiple perpetrator sexual assault. Each of these cases excluding a suspect represents an individual who was erroneously identified, but DNA analysis findings excluded the identified suspect.

When considering the implications for testing sexual assault kits, it is important to recognize that justice is the ultimate goal. Testing sexual assault kits uses science to aid in the establishment of justice. Sexual assault kit testing transcends accusations and labeling to provide factual evidence supporting the claims of the innocent, whether they are identified as victim or suspect. Additionally, the liberation of an unjustly accused suspect promotes the correct identification and conviction of the responsible perpetrator.<sup>3</sup> This research supports the inclusion of suspect samples in sexual assault kit testing to promote justice for all. Thus, mandated testing of sexual assault kits promotes justice for victims of sexual assault and innocent suspects alike.

#### Reference(s):

1. Peterson J., Johnson D., Herz D., Graziano L., Oehler T. Sexual assault kit backlog study. Washington, DC: *The National Institute of Justice*. 2012.
2. National Institute of Justice. *National best practices for sexual assault kits: A multidisciplinary approach*. 2012:1-123. <https://nij.gov/topics/law-enforcement/investigations/sexual-assault/Pages/national-best-practices-for-sexual-assault-kits.aspx>.
3. Goodman-Williams R., Campbell R., Sharma D.B., Pierce S.J., Feeney H., Fehler-Cabral G. (2019). How to right a wrong: Empirically evaluating whether victim, offender, and assault characteristics can inform rape kit testing policies. *J Trauma Dissociation*. 2019;20(3):288-303. doi:10.1080/15299732.2019.1592642.

#### Justice, Suspect Exclusion, Sexual Assault Kit



## E104 Giving a Voice to Male Rape Victims Through Novel Short Tandem Repeat (STR) DNA Findings

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**Learning Overview:** After attending this presentation, attendees will have increased understanding and competency on the subject of male rape victims and DNA analysis findings from sexual assault kits of male victims.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing new research findings regarding male rape victims and their sexual assault kit DNA analysis findings to inform knowledge, practice, and policy for researchers, forensic examiners, forensic scientists, law enforcement, and criminal justice system professionals.

Traditionally, rape has been considered to occur between a male perpetrator and a female victim. However, sexual assault is a crime that affects all genders. Although the majority of rapes are male to female, current findings indicate that 1 in 71 men will be raped in their lifetime.<sup>1</sup> While the psychological effects of male victim rape and its underreporting are well documented, very little has been reported regarding DNA findings from sexual assault kits of male victim rapes. After an intensive search for earlier publications concerning the topic, only three articles were found to have relative correlation to this topic. Two of the three articles date back to 1978 and 1982 and the studies focused on spermatozoa findings in the anal cavities of female victims.<sup>2-4</sup> Current best practice is to obtain STR DNA profiles from sexual assault kit samples to enter into the Federal Bureau of Investigation (FBI) Combined DNA Index System (CODIS). This study methodology is an exploratory, retrospective design to identify male rape victims from a sample size of 5,442 victims who received sexual assault forensic examinations with sexual assault kit evidence collection.

Approximately 5% of the victims in this study were male ( $N=246$ ). Male victims were found to have less non-anogenital and anogenital injury, although they reported more violent acts by suspect(s). Male victims reported that ejaculation occurred less often during the assault than female victims. Male victims were found to have more physical or mental impairments, increasing their vulnerability for sexual assault. Male victims had substantially lower development of STR DNA profiles and CODIS-eligible DNA profiles of perpetrator ( $p=.007$ ). In fact, female victims were 40% more likely to develop a CODIS-eligible profile from sexual assault kit DNA analysis than male victims.

Due to low STR DNA profile yields and increased targeting of mentally impaired or otherwise vulnerable male victims, we must improve our response to male victims to ensure justice to all victims of sexual assault.

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1. Black M.C., Basile K.C., Breiding M.J., Smith S.G., Walters M.L., Merrick M.T., et al. *The National Intimate Partner and Sexual Violence Survey: 2010 Summary Report, 2011*. Centers for Disease Control and Prevention, National Center for Injury Prevention and Control. [http://www.cdc.gov/ViolencePrevention/pdf/NISVS\\_Report2010-a.pdf](http://www.cdc.gov/ViolencePrevention/pdf/NISVS_Report2010-a.pdf).
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### Males, Rape, STR DNA



## **E105 Homicide by Unspecified Means: The Importance of a Forensic Pathologist and Anthropologist Teamwork Approach in Death Scene Investigation and Postmortem Analysis**

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**Learning Overview:** After attending this presentation, attendees will recognize deaths that meet the diagnostic criteria of homicide by unspecified means and will highlight the importance of the forensic pathologist and anthropologist leading the death scene investigation and postmortem analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance of a teamwork approach between the forensic pathologist and anthropologist in the medicolegal death investigation and postmortem analysis in suspected homicide by unspecified means-related deaths.

A complete medicolegal death investigation includes a death scene investigation, postmortem examination, and ancillary studies. A death that occurs under objectively suspicious circumstances, when there is no anatomic cause of death, no toxicology cause of death, no environmental, circumstantial, or historical cause of death, or when a more specific cause of death cannot be suggested, the terms “homicide by unspecified means,” “homicidal violence,” and “homicidal means” are suggested as an appropriate cause of death.<sup>1</sup> Commonly, deaths that fulfill this diagnostic criterion involve decomposed and skeletonized decedents that require additional forensic investigation and analysis. Reported here are two cases of skeletonized females that were found buried in wooded areas after their husbands killed them.

A 44-year-old female was missing for approximately five months when her husband was found dead in his residence. He hanged himself and left a confession note and map that detailed he choked the decedent to death and buried her body the next day. Based on the map, the female was found in a shallow grave in a densely wooded area. A team from the medical examiner’s office that included the forensic pathologist, two forensic anthropologists, and a medical examiner investigator led the death scene investigation of the female, which revealed predominantly skeletal remains. At the morgue, the forensic anthropologists performed a skeletal analysis, which revealed a peri-mortem defect of the right side of the skull; remote, healing, or healed fracture of the maxilla and right palate; and no fractures of the unfused hyoid bone. While the examination of the body did not demonstrate a specific cause of death, given the circumstances, the decedent’s cause of death was homicidal means, probably of asphyxia due to strangulation, and the manner of death was homicide.

A 32-year-old female was last known alive in 2010. In 2018, the decedent’s husband led law enforcement to a wooded area where he buried the decedent after killing her. He confessed he stabbed the decedent in her neck. A team from the medical examiner’s office that included the forensic pathologist, two forensic anthropologists, a forensic anthropology coordinator, and a medical examiner investigator led the death scene investigation of the female’s skeletal remains. At the morgue, the forensic anthropologists performed a skeletal analysis, which was most significant for sharp force trauma to the right sides of cervical vertebra 7 and thoracic vertebra 1, representing a minimum number of one impact, consistent with a knife cut injury of the lower aspect of the right side of the neck. Given the circumstances, the cause of death was homicidal means, including sharp force injury of the neck, and the manner of death was homicide.

When the diagnostic criteria for homicide by unspecified means under objectively suspicious circumstances of death are met, which includes a body deliberately hidden from view, attempt to conceal evidence, and scene findings suggestive of antemortem injury not verifiable by autopsy, it is recommended that the forensic pathologist and anthropologist be present and lead the death scene investigation and that a full autopsy with skeletal analysis is completed.<sup>1</sup> The presence of both the forensic pathologist and anthropologist at the death scene may serve to locate the decedent with minimal disruption if buried, preserve potential evidence, determine human remains versus non-human remains and objects, appreciate possible postmortem animal activity, and identify possible peri-mortem trauma. A full autopsy with a skeletal analysis is recommended because, as highlighted in the second case reported here, the forensic anthropologist documented sharp force trauma of the vertebrae that was consistent with the confession given, and can provide specific, scientific cause of death information in an otherwise homicide by unspecified means-related death.

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### **Homicidal Means, Investigation, Analysis**



## E106 Drowning: The Silent Death

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**Learning Overview:** After attending this presentation, attendees will be aware of correlations between age and drowning locations within Harris County, TX, to help bring awareness to the issue and open a discussion about drowning prevention.

**Impact on the Forensic Science Community:** This presentation will inform the forensic science community by demonstrating the prevalence of drowning, specifically pool drowning, of children aged 12 years and under. Additionally, this presentation will address how misconceptions about drowning can play a contributory role and will provide information to combat these misconceptions.

The Harris County Institute of Forensic Sciences in Houston, TX, averaged approximately 4,559 medicolegal cases each year between 2013 and 2018. Approximately 1,500 medicolegal cases each year from 2013 to 2018 had a manner of death classified as accident. Approximately 4.3% of the accidental deaths each year were drowning, which is a total of 334 drownings classified as an accident between 2013 and 2018.

To focus on the data, the 334 drownings were separated by location: bathtub, open water, and pool. Once the cases were separated by location, trends were noticed in the age of the decedents. Through further analysis, the data revealed correlations in age range and location of the drownings. When examining the age variable in pool locations, a sharp peak was noted in children aged 12 years and under. A large broad peak in open water drownings of individuals in their early 20s into their mid 30s was also observed. Last, bathtub drownings revealed a peak of individuals in their mid-50s to mid-70s. After comparing the number of drownings in each location to the ages of decedents, a pattern was noted among child drownings and location. Approximately 9% of the total 76 bathtub drownings were children and approximately 12% of the total 128 open water drownings were children. In contrast, approximately 51% of the total 130 pool drownings were children.

The data collected revealed that in Harris County, children aged 12 and under are more likely to drown in pools than in any other location, such as bathtubs or open water (i.e., bayous, rivers, ponds, or lakes). Analysis of the 469 cases classified as drownings between the years of 2013 and 2018 revealed that 130 drownings occurred in pools. Of the 130 pool drownings, 66 (approximately 51%) were children age 12 years and under. Upon further analysis of the 66 child drownings, it was noted that 48 (approximately 73%) were children age 3 years and under. The initial 51% of child drownings, age 12 years and under, is three times greater than the number of child drownings observed in bathtubs and open water combined, which was 22 (approximately 11%). Not only do the majority of child drownings occur in pools, but approximately 76% of the 66 child drownings occurred at private residences. It was also noted that of the 15 open water child drownings, 6 were in ponds or man-made bodies of water located on the property of the family.

Child drownings are more likely to occur in pools at private residences than at any other location in Harris County. To combat this, it is imperative that adults be more alert and aware of their child's location, especially when they have a pool or other body of water in their backyard. Some ways to provide protection include fencing around pools or door alarms in the house to alert someone when a door has been opened. Children are very resourceful, even at young ages, so taking extra measures goes a long way toward ensuring their safety.

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### Drowning, Children, Accident



## E107 Let's Solve It: Designing an Interactive and Engaging Online Forensic Science Laboratory Course

Gina Londino-Smolar, MS\*, Indiana University - Purdue University Indianapolis, Indianapolis, IN 46202

**Learning Overview:** After attending this presentation, attendees will: (1) explore the design process of an online course; (2) create new learning assessments for students in an online environment; and (3) discover how online lab experiences can be formed through virtual and hands-on experiences.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by displaying the development process of an online laboratory course in forensic science. Online courses are the norm on most college campuses, even courses online in forensic science, but what about laboratory coursework online? This presentation will enable attendees to learn about the design process of a completely online laboratory course in forensic science. The course material and learning assessments will be shared, which can inspire attendees in their own online course development.

There has been a rise in the percentage of students that complete course work online. With that, there is an increase of science laboratory experiences being offered in a fully online environment. These experiences range from fully virtual laboratory exercises to kitchen chemistry kits to customized kits for traditional laboratory exercises. Fully online laboratories are developed for non-science major courses to increase enrollment. The goal of developing an online laboratory course is to offer a similar experience to that of a face-to-face course while engaging the student in the content.

Designing a hands-on online laboratory experience has advantages for both the university and the student. The university cost associated with online laboratory courses is much lower, both with space and equipment. Online laboratory courses that use at-home kits will not have the increased cost of laboratory space and teaching assistants, while virtual labs can cut costs without the extensive supply need.<sup>1</sup> Face-to-face laboratory fees can be allocated to purchase at home laboratory kits. This allows online students to have a similar experience as face-to-face students at a much lower price to the university without raising student cost.

A hands-on laboratory experience allows students to engage in their own environment. Doing hands-on experiments can enhance students' appreciation of science in their own lives.<sup>2</sup> It was also found that a virtual environment can develop a student's deeper understanding of the context through the experience.<sup>3</sup> This includes an understanding of theoretical concepts and confirming hypotheses, which leads to increasing students' motivation to learn. However, there are some disadvantages, which were a lack of skill with chemical and lab equipment as well as observation of reactions.<sup>4</sup> Therefore, as at-home labs are developed, demonstrations along with snapshots for steps throughout the lab process will be captured and shared with students. This will help students through the lab procedures and with equipment used during the lab. For more complicated labs, virtual laboratory experiences are chosen. Specific virtual labs will be highlighted along with an explanation of why these specific laboratory exercises were designed virtually.

This course was developed from a course currently being taught in the classroom and converted to be offered solely online. Students will have the same learning objectives regardless of the environment in which the course is completed. The different types of laboratory exercises specifically developed for the online course, which include virtual labs similar to the face-to-face lab experiments as well as lab experiments from a commercial kit, will be shared. Students will use a kit with equipment and specific directions or demonstrations on how to complete the laboratory exercises for the hands-on experiments. Virtual experiments were designed to simulate the same type of labs done in the face-to-face sections that could not be replicated in the kit. A comparison of the lab experiments between both environments and the design process will be shown.

Throughout the presentation, the lessons learned during the development process, how the course will be implanted at this university, and future goals of the course will be discussed. Attendees will take away useful design strategies when developing an online lab course by comparing both hands-on and virtual lab exercises and the assessment plan to have the students think critically and engage in the material.

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### Education, Online, Laboratory



## E108 High-Impact Learning Within a Project-Based Learning Course

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**Learning Overview:** After attending this presentation, attendees will understand how high-impact learning activities can be implemented into forensic science courses, even courses that are heavily activity based.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the pedagogical benefits of high-impact learning in a forensic science curriculum.

The Association of American Colleges and Universities in 2008 published a report on High-Impact educational Practices (HIP) and their importance to undergraduate education.<sup>1</sup> Effective HIP activities share several commonalities: expense of considerable time and effort, faculty and peer interaction, experience of diversity, experience feedback, and learning in different settings (i.e., outside of the traditional classroom lecture). HIP is all about focusing student engagement in their own learning. Ten proven HIP activities have been identified. Included among these are: (1) collaborative assignments and projects, and (2) undergraduate research.

In 2012, in response to a university-wide initiative, the Anthropology and Sociology Department at Western Carolina University adopted a requirement that all majors participate in an Engaged Learning Experience (ELE). This requirement could be fulfilled in a number of manners, including enrolling in an ELE-designated course. Anthropology 401, Bone Trauma and Modification, is a Project-Based Learning (PBL) themed course in the forensic anthropology curriculum.<sup>2</sup> Students engage in group activities involving the predetermined replication of bone traumas using non-human animal bone. These replications are carried out in small groups of three to five students. In 2018, this course was designated an ELE course. This meant that, in addition to the regular PBL activities performed by all students in the course, the 13 ELE participants were required to perform a separate and unique bone trauma replication project. They were also required to make a public PowerPoint® presentation of their results and submit a written report.

HIP is by its nature time- and labor-intensive not only for students but also for faculty. This creates the potential for student and especially faculty participant overload.<sup>3</sup> As Anthropology 401 is a laboratory course, introducing an added laboratory component in the form of the ELE activity compounded the logistics of time and space in the laboratory. Essentially, an HIP course had added to it a second HIP component. One solution to avoid synergistically intensifying time and space requirements was to make the ELE activity group-optional. Participants could perform their activity individually or in groups of up to four individuals. The latter was strongly encouraged and only one student chose to work on their own.

Each year, the ELE projects are internally reviewed by the department. This review process includes direct observation of the ELE public presentation as well as the physical products of the student-engaged research. Of the 13 Anthropology 401 projects submitted in 2018, 12 met and 1 exceeded expectations for quality, scientific rigor, and meeting the spirit of engaged learning. The one project that exceeded expectations was submitted by the single student participant.

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### Education, Experiential, Learning





## E109 Engaging Freshman Undergraduate Students in Forensic Science Research to Improve Science, Technology, Engineering, and Math (STEM) Retention

Kristi Bugajski, PhD\*, Valparaiso University, Valparaiso, IN 46383

**Learning Overview:** After attending this presentation, attendees will have been introduced to two different grant programs that are supporting forensic science undergraduate research. An outline of the programs and the types of projects students have worked on will be presented. A discussion of how other institutions could run similar programs will be part of the discussion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that there is great value in students having an authentic research experience in terms of the student's skill development and retention at a university. The impacts are intensified when students are recruited into laboratories in their freshman year.

Previous research shows there is great value in an authentic research experience for undergraduates.<sup>1</sup> Other studies have documented the importance of undergraduate research in developing student's scientific knowledge, basic laboratory skills, self-confidence, and personal motivation.<sup>2,3</sup> These improvements are even more defined in traditionally underrepresented groups, so incorporating these students in the lab helps with Valparaiso University's (VU) commitment to diversity.<sup>3</sup>

Valparaiso University has two current grant programs that are aimed at improving freshman-sophomore retention in STEM majors by incorporating students in undergraduate research labs the first semester of their freshman year. Forensic science is one of the areas that students can choose to focus their research on, and it is a subject that most find interesting and accessible. The Freshman Research Engagement in Science (FRESH) program has been funded through the Indiana Space Grant Consortium for \$14,999 annually over the past four years. The grant provides a \$1,000 research stipend to the chosen students, along with supply/travel money for their project. The students are committed to participating in a research laboratory for two semesters, both in their freshman year. The grant does not provide money for programmatic activities outside of research and conference travel. The Establishing Practices Integrating Commuters (EPIC) is a National Science Foundation (NSF) Award in the sum of \$999,991. The grant provides a sizeable four-year scholarship (average of \$5,300 annually) in addition to supply and travel money. The aim of the grant is to use research laboratories as a basis for social integration to improve retention of commuter students. EPIC has funding for program activities, such as monthly meals, field trips, industry trips, a yearly retreat, and a writing circle.

Students in both programs have participated in forensic science research projects. The projects they have worked on are: (1) diurnal oviposition of blow flies, (2) characterizing the volatiles associated with decomposition, and (3) blow fly oviposition on wounds vs. natural openings. Only one forensic science student has not continued in their research lab after their freshman year ended. These students have presented the results of their research in a multitude of venues, including departmental colloquiums, VU's undergraduate research symposium, and the Indiana Academy of Science annual meeting. They have participated in the research ideas, experimental design, data collection, insect identification, data analysis, and both written and oral presentations.

Typical freshman-sophomore retention in a student's chosen STEM major at VU is around 65%. Both the FRESH and EPIC programs have shown significant improvement in retention at the 0.05 level. FRESH has retained 87.5% of students in their discipline after their first year and EPIC has retained 87% of students in the same period. Forensic science research has had an important role in recruiting and retaining students for both of these programs.

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### Forensic Science Research, Freshman, Undergraduate Research



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## E110 Forensic Science Distance Education: Trials, Tribulations, and Triumphs

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**Learning Overview:** The goal of this presentation is to make attendees aware of the current trends in forensic science education and how the demand for online distance education within the forensic sciences is rapidly growing. After attending this presentation, attendees will better understand the fundamental difficulties associated with building a successful distance education program, learn methods to overcome these difficulties, and hear about the student successes associated with these programs. New ways to integrate scene photos, autopsy photos, and case studies within the course will be discussed as well as the implementation of new, in-person, hands-on laboratory class electives.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating attendees about the new trends in forensic science higher education. If these same concepts are applied toward continuing education for actively working professionals—a plan that is currently in progress—it will serve to improve the quality of distance-learning continuing educational opportunities available to professionals working within the forensic sciences.

Unique to Florida, but mirrored in other states, is the Earnhardt Family Protection Act, which overrides Florida's open public records laws as they relate to autopsy and crime scene photographs, preventing the copying, distribution, or viewing of these photographs. Violation of this law is a third-degree felony and violates Florida State Statute 406.135. As many of the faculty within these online programs have built their forensic careers in Florida, this law may have proved prohibitive to providing students with the type of engaging case studies needed to further their education. However, special permissions and online security techniques have been implemented to allow the use of the photographs to provide students with the highest-quality educational experience possible. Techniques that are otherwise difficult to describe online can be supplemented with optional hands-on lab classes that give students the opportunity to immerse themselves in an intensive course that complements the online course they have previously taken.

An additional component of learning is though discussion and debate with fellow students, which is often overlooked or absent in online education. Methods to encourage and stimulate discussion are integrated within each course to ensure students receive a well-rounded education. This model has, thus far, proven extremely successful as demonstrated by the online programs performing far beyond the initial market projections for student engagement. If these same concepts are applied toward continuing education for actively working professionals, a plan that is currently in progress, it will serve to improve the quality of distance-learning continuing educational opportunities available to professionals working within the forensic sciences. Offering a myriad of courses designed for the working professional (i.e., courses that may be taken on a flexible schedule from anywhere the student has computer access and an internet connection), students and participants at any level of education and experience, from the newest beginners to the seasoned professionals, may find courses tailored to their specific needs and offered either fully online or supplemented with in-person hands-on learning.

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### Education, Distance Learning, Forensic Science



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### E111 Using Online Learning and Gamification to Enhance Reasoning Skills

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**Learning Overview:** After attending this presentation, attendees will understand how and why a reasoning skill curriculum was created for forensic science students.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance of improving reasoning skills for forensic science students by incorporating online learning and gamification.

The goal of this research was to enhance students' reasoning skills using online content and gameplay. Curriculum used in the online modules and the modified version of Clue® were created by a student and a professor. Student involvement in curriculum design allows students to modify content to their own predisposition. Allowing students to have a say in what they learn and how may create a more engaging environment for education, increasing the motivation of students to learn. As a student, taking on the role of creating content and assessments assisted in a deeper understanding of teaching pedagogy.

Using the Online Learning Initiative (OLI) environment, modules focused on improved reasoning skills in the sciences were developed. Modules were equipped with videos, definitions, and activities to provide diversified means to grasp the content. The modules combined with the game were implemented in a flipped-classroom setting where students completed the online modules, then applied their knowledge while playing the game. Flipped-classroom models consist of lectures, online learning, and other various learning interphases outside of the classroom. This enables students to apply their knowledge in the classroom where thinking errors can be corrected and automatic feedback is feasible and encourages educators to be more of a mentor than a teacher.

Students were asked to complete the online modules at their own pace within a week timeframe. The primary analysis compared pre-test versus post-test scores to assess students' knowledge gain. Next, a group of students played a modified card-based version of Clue®. Only some of these students had completed the online modules. Students were asked to play the game once without any further instruction. For the second game, students were asked to play again and complete a worksheet to track their thoughts and identify reasoning processes. Analysis of the game included a comparison of answers between those who completed the online modules and those who did not. This research demonstrates how online learning and gamification can enhance reasoning skills.

Results indicate that students are learning the different types and modes of reasoning through the online content. Additionally, students who have completed the online modules prior to playing the Clue® game were better able to identify their own reasoning processes while playing the game. Overall, this work provides an engaging format to teach reasoning skills to forensic science students. Furthermore, the interaction between an educator and a student enhances the design process and develops new skills for the student.

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#### Education, Reasoning, Learning



### **E112 Crossing the Border Between Academia and Forensic Science Practice: Managing a Successful Student Internship Program in an Integrated Medical Examiner Office and Crime Laboratory**

*Jasmine M. Jefferson, MS\*, Harris County Institute of Forensic Sciences, Houston, TX 77054-2001; Michal L. Pierce, MS, Harris County Institute of Forensic Sciences, Houston, TX 77054*

**Learning Overview:** The goals of this presentation are to: (1) explain the mutual benefits in maintaining a student internship program at a forensic science agency; and (2) illustrate the elements in a successful internship program at an integrated medical examiner office and crime laboratory.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science program by helping attendees gain competence in explaining how a student internship program can mutually benefit a forensic science agency and forensic science students and in illustrating the system to maintain a successful student internship program.

In an effort to bridge the border between academic study and forensic science practice, there has been an observed increase in the number of collegiate programs with a student internship requirement as part of the forensic science curriculum. While many students seek a forensic science internship as an introduction into the field of forensic science, other students purposefully use internship programs as a stepping stone to future employment, establishing contacts, and creating connections for on-going research interests. Allowing students to crossover into the day-to-day functions in a laboratory discipline as part of their curriculum provides an invaluable experience to integrate their academic foundations with real-life experience and to plant seeds for relevant research. Moreover, maintaining an internship program allows eager and competent students to temporarily expand the workforce in a forensic science agency and perform work on various research projects, providing mutual benefits for both the student and the agency.

Managing a successful student internship program at an integrated medical examiner office and crime laboratory is a labor-intensive task, but is essential to exposing students to forensic science culture. As a priority, most forensic science agencies manage a large caseload and continually strive to meet turnaround time objectives. Although managers may identify areas throughout the agency that could benefit from research and development, they may be unable to dedicate lab analysts to develop validation plans or perform the research functions. This is where interns can assist. However, intern recruitment, applicant review, and mentorship require staff and a developed system. Furthermore, once students are accepted to an internship program, they require consistent oversight, a structured schedule, professional development guidance, and end-of-program activities that are critical to providing the student with a comprehensive intern experience and achieving a focused outcome for the agency.

Yearly, the Harris County Institute of Forensic Sciences (HCIFS) opens intern positions in numerous forensic science disciplines, including forensic investigations, forensic anthropology, forensic entomology, histology, forensic genetics, trace evidence, forensic toxicology, drug chemistry, firearms identification, and victim's assistance, as well as forensic imaging and administrative services. The HCIFS employs a full-time Training & Development Manager to manage the student programs, including the internship program. The Quality Management Division staffs the Training Manager, who is integral to the success of maintaining the ten-week, full-time summer internship program, and the additional intern program needs throughout the year.

The Training Manager maintains relationships with local colleges and universities as a regular source of student interns, as well as handling recruitment and outreach to a variety of schools. Application processing and screening is filtered through the Training Manager, who also tracks the applicants for each discipline and can initiate additional recruitment efforts for disciplines with multiple project needs. A trained mentor and special project is assigned to each intern once applicants are selected for and accepted into the program. Upon their arrival, the Training Manager provides an orientation to onboard all students to the agency. The Training Manager creates and maintains a formal internship program schedule with pre-scheduled professional development sessions for the interns, which has proven to be one of the most meaningful aspects of the HCIFS student-training program in further preparing students to cross over into the professional forensic science workforce. Additional activities unique to the HCIFS Internship Program, such as an autopsy viewing or forensic investigations field call ride-along, may also be scheduled to enhance the intern experience. At the end of the program, each intern provides a formal presentation to HCIFS managers and staff to summarize the project completed during the program. Finally, the HCIFS Training Manager facilitates the intern performance evaluation process and periodic progress reports with each mentor to ensure administrative documentation is maintained as part of the internship program. In 2019, the HCIFS received 35 intern applications from qualified students; 13 interns were selected for the summer internship program from six different universities.

#### **Forensic Science Internship, Student Engagement, Student Intern**



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### E113 Forensic Sciences Learning for Victims of Enforced Disappearances

*Carlos A. Gutierrez, MS\*, Chaminade University of Honolulu, Honolulu, HI 96816*

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**Learning Overview:** The goal of this presentation is for attendees to learn about an innovative teaching program in basic forensic sciences and crime scene investigation developed for the relatives of victims of enforced disappearances that happened in Sinaloa State, Mexico. This program has been developed to be offered online from Chaminade University of Honolulu, HI, to its recipients in Culiacan, Sinaloa State, Mexico, in Spanish.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that people without any background or knowledge of the forensic sciences and crime scene investigation field can have the ability to address and process a crime scene. This educational program can be utilized for other groups of victims of enforced disappearances around the world, in different languages.

Enforced disappearances are a major issue that several nations have faced throughout history and that have left a problematic number of people declared “missing” around the world. An enforced disappearance is the kidnapping process of a person by an unknown individual/organization with the purpose of acting outside the margins of the law. Oftentimes, enforced disappearances entail the assassination of the kidnapped person, whose body is usually discarded, leaving the feeling that the victim has faded away from the face of the earth. Enforced disappearances have occurred in different contexts throughout history; some of the most remarkable contexts are dictatorships, armed conflicts, and drug trafficking. These illegal actions have been used to spread terror in countries and regions around the world, such as Sri Lanka, Syria, and Mexico, countries where enforced disappearances have become a serious problem not only for the victim and governments but also for close relatives.

Currently, drug trafficking is considered one of the most important contexts in which numerous people are declared “missing,” and Mexico ranks among the countries with the highest number of enforced disappearances. Groups of close relatives of enforced disappeared people have formed throughout Mexico, initially with the main goal of supporting the close relatives of the “missing,” but subsequently with the purpose of searching for any kind of physical evidence that can be provided to the prosecutor’s office to help with the investigation of these cases. One of these groups is Sabuesos Guerreras (Warrior Hounds), a not-for-profit organization from the city of Culiacan, in Sinaloa, Mexico. This group is composed mainly of women whose beloved husbands and/or sons have been victims of enforced disappearances, who for years, in addition to supporting each other sociologically and economically, have searched for physical evidence in different open fields of the state.

These acts have inspired the creation of an innovative training program in basic forensic science with the goal of being able to use the knowledge acquired through this program in their own relatives’ cases. This training program consists of a ten-class online course, focusing on: “Crime Scene Investigation,” “Analysis and Preservation of Physical Evidence,” and “Management of an Organization for Victims of Enforced Disappearances.” In this program, participants are assessed before the course begins and after it ends using the same evaluation instrument.

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#### **Education, Enforced Disappearances Victims, Online Teaching Program**



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## E114 Curriculum and Accreditation Pertaining to Crime Scene Investigation Education

*Mark Vecellio, MFS\*, Methodist University, Fayetteville, NC 28311; Erick P. Bryant, MFS\*, Colorado Bureau of Investigation, Arvada, CO 80002*

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**Learning Overview:** After attending this presentation, attendees will have gained insight into the current state of accreditation relating to crime scene investigation education and ideas concerning development of a model program.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through discussion of potential benefits of accreditation and oversight of university crime scene investigation programs. Additionally, a proposed framework supporting an effective educational Crime Scene Investigator (CSI) program will be presented.

Traditional university forensic science programs generally prepare students for work in forensic laboratories. Such programs may be accredited through the Forensic Science Education Programs Accreditation Commission (FEPAC), thus helping to standardize and maintain assurance of quality and relevant education. FEPAC accreditation, logically, focuses on the sciences. CSI work, however, is often completed by investigators who are not formally trained scientists nor have completed degrees in a scientific field. Many universities have developed programs focusing on preparing students for CSI or general investigative work; however, these programs are quite variable in terms of curriculum, the methods in which educational content is delivered, and quality.

The primary objective of this presentation is to raise awareness and initiate dialogue relating to accreditation of CSI educational programs. Though CSI work, especially at entry level, does not require the same depth of understanding of the sciences as does forensic laboratory work, it does require thorough knowledge and technique mastery of identification, collection, initial processing, and storage of physical evidence, as well as the ability to request relevant forensic exams of physical evidence that is recovered. Some research, and a wealth of professional anecdotal experience, suggests that evidence is routinely overlooked, improperly processed, or not submitted to forensic laboratories for appropriate testing, thus severely limiting the full capabilities of forensic science. It is thought that quality college education would naturally help correct this problem. Accreditation would help ensure students receive quality, rigorous education.

The second objective of this program is to encourage dialog concerning program objectives and coursework for CSI-related curriculum. A model program will be discussed. This program has undergone over ten years of refinement and development and is based upon training methods used by federal and state law enforcement training centers. The program grounds students in the basic science and the necessary hands-on skills to expertly locate, document, identify, process, collect, protect, and analyze many types of forensic evidence in support of felony investigations. This education/training prepares students for the potential to pursue advanced degrees as well as employment within the forensic science and law enforcement/investigative professions. In addition, the curriculum content is consistent with the primary national certification program.

Program coursework, in addition to general education requirements including life and physical science, consists of a theoretical foundation of courses, including the Introduction to Forensic Science, the Investigative Process, Drugs of Abuse, Homicide and Death Investigations, Forensic Profiling, and Forensic Firearms. Students also complete a series of lab-based courses, including Forensic Photography, CSI, Advanced CSI, and Crime Scene Reconstruction. Students engage in approximately 100 hours of practical exercises in addition to hands-on experience in internships.

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### Education, Accreditation, Crime Scene Investigation



### E115 Taking Science Behind the Walls: Side-by-Side Learning With Students and Incarcerated Individuals

Susan M. Gurney, PhD\*, Drexel University, Philadelphia, PA

**Learning Overview:** After attending this presentation, attendees will have greater insight into the Inside-Out pedagogy and how it can be used to enhance learning and understanding within a science course taught in a correctional facility.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a novel pedagogical approach that may be incorporated into an undergraduate or graduate forensic science curriculum and which juxtaposes the course content with a criminal justice context.

Inside-Out is a national program that brings together college students and incarcerated individuals to learn side-by-side through facilitated discussion. The first Inside-Out class started in 1997, Temple University in Philadelphia, PA, and as of 2019, more than 200 correctional facilities have hosted over 35,000 inside and outside students.<sup>1</sup>

Initially, the Inside-Out courses addressed topics surrounding criminal justice, but have expanded to cover many topics within the humanities and social sciences. In 2019, Drexel University taught the first science-based Inside-Out course, The Science of Science Fiction, bringing together 12 outside students from different majors (Biology, Biomedical Engineering, and Communications) with 14 inside students from Curran-Fromhold Correctional Facility. The course met for two hours weekly, during the ten-week quarter term. The topic of science fiction was specifically selected to ensure that it was accessible to students from different educational and scientific backgrounds. It also allowed discussions on many different aspects of science, including evolution, the physics of time travel, and editing of genomic DNA, while also allowing discussions on such themes as societal structure, prejudice, and justice.

The role of the instructor in the classroom setting is as a facilitator to the discussions and learning. Through group work and class discussions, the aim of the program is for the students to learn from each other entirely through discussion, without any traditional lecturing. This was adapted slightly to introduce some more complex scientific concepts, to introduce recent scientific advances. The course was designed around two novels, *The Time Machine* and *The Genius Plague*; one comic, *Astounding X-Men*; and one television show, an episode of *Doctor Who*. Students were given weekly reading and assignments to complete. In class, students would start by working together to outline the scientific concepts covered in the assigned reading from that week. From the concept, they would select two or three for further discussion. The instructor prepared “pop-up” lectures lasting three to five minutes on two or three of the main themes, providing more context for the science content and the current understanding. Group activities allowed students to work together to draw images of characters, design technology that may have been used in the reading, or to discuss specific quotes in more detail. Class discussion allowed students to share their views and their own understanding and insights of science with each other. Over the course of the ten weeks, it was evident that the Inside-Out model works well with a science-based curriculum. The students were fully engaged in the pre-class readings and very active within the classroom. The most successful part of the class was the class discussions, which were varied in their topics, but were always focused on science and the themes within the readings. This course helped to break down the barriers between inside and outside students, generating an active learning community over the course of just ten class meetings.

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**Education, Facilitated Discussion, Incarceration**



### E116 The Lessons Learned in Teaching Forensic Toxicology From “Wet Lab” to “Lab Bench”

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**Learning Overview:** The goal of this presentation is to identify the elements and challenges in developing the full arc of an experiment from the field to the bench that involves human participants and the regulatory requirements.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying solutions to challenges in developing robust, engaged, field-to-bench education modules for students learning the practice of forensic toxicology.

The Forensic Science Education Programs Accreditation Commission (FEPAC) -accredited Department of Forensic Science (DFS) at Virginia Commonwealth University (VCU) has a three-year average of 320 undergraduate majors and 20 masters-level graduate students. Since the inception of the DFS as a stand-alone academic unit in 2000, a three-credit graduate lecture course in forensic toxicology has been a requirement for master students on the Drugs and Toxicology Track. An accompanying laboratory course was established Spring 2019 as a result of policy shifts that enabled faculty to build and teach this course. The laboratory course focused on standard analytical techniques encountered in the forensic toxicology sub-disciplines of urine drug testing, postmortem toxicology, and Driving Under the Influence (DUI) testing. The course was co-taught and utilized the combined experience of both faculty members to develop the laboratory module as the course was being implemented. In a collaboration with the course faculty and with the generous help of VCU's Police Department (VCU PD), a laboratory module was developed that would take the student from the field to the laboratory. The purpose of this laboratory module was to have students perform the analysis of blood for volatile compounds, including ethanol, improve their understanding of the effects of ethanol and of zero order elimination, and to observe the Standardized Field Sobriety Test (SFST) by trained Law Enforcement Officers (LEO) in a safe environment to facilitate questions and engagement.

The development of this laboratory module involved VCU PD running a wet laboratory re-training session for their officers to review and practice SFSTs. The wet laboratory was conducted as required by the accreditation standards for the law enforcement academy. Volunteer drinkers were enlisted. The volunteers were given Preliminary Breath Alcohol Test (PBAT) at routine intervals before and after the SFST was administered. An on-site registered nurse collected blood from the volunteers prior to drinking and immediately before the SFST was conducted in compliance with requirements of the DFS's Institutional Review Board (IRB) protocol (HM20002931 CR3) for de-identified collections. Students were provided a lecture prior to the wet laboratory regarding DUI laws and the SFST by an instructor in the VCU PD Police Academy. The student observed the SFST performed by LEO as re-training and review. The collected PBT, blood specimens, and the SFST results recorded by the LEO were de-identified and given to the students for bench analysis. The student used the PBT results to generate an ethanol elimination curve, and they analyzed the blood samples by headspace Gas Chromatograph with Flame Ionization Detectors (GC/FID). The students then correlated the results of the PBT, Blood Alcohol Concentration (BAC), and the SFST results.

#### Education, Toxicology, Wet Lab





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### **E117 High Tech, High Touch Learning for High School and Undergraduate Forensic- and Crime Scene-Related Fields of Study**

*Charla Skinner Perdue, MS, MFS\*, Florida State University Panama City Campus, Panama City, FL 32405*

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**Learning Overview:** The goal of this presentation is to demonstrate a model in which experiential learning can be utilized in high school and undergraduate forensic- and crime scene-related fields of study by integrating traditional classroom and online learning for a tech-enhanced learning modality.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the benefits of educators developing experiential learning courses and of students participating in career-building experiences before graduation.

Online education is beneficial and continues to grow in popularity. Graduates from forensic and crime scene-related online programs frequently leave with an understanding of key concepts but with little ability to demonstrate the skills and techniques they can explain. By requiring experiential learning courses in a mostly distance-learning program, students graduate with relevant real-world experience on their resume and are better prepared for the job hunt and workplace. Students are buoyed by the ability to point out to potential employers that they know about a particular subject and have completed the processing, testing, analysis, etc.

There are many challenges when it comes to incorporating hands-on learning into a mostly online educational program. This presentation will detail how the Florida State University (FSU) faculty have developed curriculum for the Crime Scene Investigation Major that uses technology to deliver lectures and labs live, online for distance learners and uses accelerated on-campus summer semesters to further develop skills. With proper planning, this strategy can be emulated by interested institutions.

This presentation will detail how students at FSU participate in introductory course work that is lecture-based to gain understating of a particular forensic discipline. This is done traditionally for face-to-face students in the classroom and Using Blackboard Collaborate Ultra, distance learners join the classroom remotely from their laptop or other mobile device. Distance-learning students are able to join in in the discussion using their microphone or the built-in chat feature.

During hands-on labs, traditional students are in the laboratory with the instructor. For the online student, a box is mailed at the start of the semester with the materials needed to participate in the lab. Like the lecture, distance learners join with traditional students from wherever they are located and participate in the lab using their own lab in a box. These technology-enhanced courses provide the underpinnings of the more intensive on-campus summer labs.

Summer labs are held in an accelerated summer semester on campus only and are required. Students travel and stay on campus for one week and attend lab Monday through Friday from 9:00 a.m.–5:00 p.m. daily. During this week-long, 40-hour lab, students receive the full lab credit that would normally be taught over a traditional 13-week summer semester. During this time, the traditional and online students have the opportunity to get to know one another personally and work together in small groups, problem solving and completing casework.

At the end of the program, students are required to participate in a Capstone internship program. This is done with a crime scene-related agency in the student's area and does not require relocation. Students must apply for the Capstone and have their agency and position approved by the program. While working at the agency, students complete weekly assignments highlighting what they are learning. Results from this program, which had its first graduates in the summer of 2017, have been obtained in post-graduation surveys and reveal alumni are at certain hands-on labs and the required internship gave them an advantage in hiring.

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#### **Education, Curriculum, High School/College**



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### E118 Chemical Murder Mystery: Two Decades of Experiences of Forensic Science Education in Delivering a Hands-On Event for 15- and 16-Year-Old Students at a University

*Stewart Walker, PhD\*, Flinders University, Adelaide, South Australia 5001, AUSTRALIA*

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**Learning Overview:** The goal of this presentation is to present experiences of writing and presenting hands-on forensic science events for school students between the ages of 15 and 16 years old. Some of the problems and pitfalls will be discussed along with some of activities that worked. Consideration will also be given to changes that have been made over the two decades of running these events and of what students expect from these events in 2020.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by relating experiences to attendees who may have undertaken, or plan to participate in, forensic science school outreach programs.

The Chemical Murder Mystery (CMM), a hands-on, day-long activity for 15- to 16-year-old students, has been run at the South Australian Branch of the Royal Australian Chemical Institute ([RACI; the equivalent of American Chemical Society) and The School of Chemistry, Physics and Earth Sciences at Flinders University, Adelaide, South Australia, over the past two decades. The basic format has been to gather the students (up to 144 per day) and split them into 12 teams with different colored T-shirts. The students then gather in a lecture theater for a presentation, “CSI: Adelaide. Forensic Fact or Forensic Fiction,” that illustrates the breadth of forensic investigations from DNA, drugs, fingerprints, soil, toxicology, etc. The presentation is interrupted by a policeman or security guard who says that the head has been found dead. The students then become crime scene investigators and go to the (reconstructed) crime scene. Two representatives from each team collect evidence from the car, the tires, the shoes, and the body of the deceased. After a short overview of the evidence collected, they go to the lab and analyze the evidence at 1 of 12 stations, then rotate stations. After the evidence has been analyzed, they congregate in the theater again, which serves as a court room. One representative from each team comes to the stand and presents what they did and what they found. The different evidence is then placed on the white boards under the appropriate suspects. At the conclusion, the students vote on who they think “dunnit” and how. This has proven popular with local schools and some who travel in from the country. At peak, there were 850 students enrolled for six days with 500 on the waiting list. This is a major event for a small department and small RACI group.

Over the two decades of training, many changes have taken place and the CMM has developed and changed to: keep relevance with the changing school curriculum; enable more students to attend; adapt to changes in students’ expectations; include input from new forensic staff; and keep up with changes in Health and Safety requirements. The initial aim of the Chemical Murder Mystery was not to attract students to Flinders University’s Forensic Program (BSc, BSc[Hons], MSc, and PhD in Forensic and Analytical Chemistry and Biology/DNA), but to encourage students to choose to continue studying science in the last two years of school by having a day of fun using science to solve a crime. This presentation will discuss some of hands-on activities that worked well with this age group but will also indicate some of the problems and pitfalls for discussion with others doing such forensic science outreach activities. It will also discuss additional potential forensic science outreach.

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#### Education, Schools, Outreach

**E119 Deceased Children Due to Trauma and the Evaluation of Non-Accidental Injuries: A Dutch Retrospective Level-1 Trauma Unit Study**

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**Learning Overview:** After attending this presentation, attendees will know the incidence of child abuse and neglect in deceased children presented at a Level-I trauma center.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing knowledge on non-accidental trauma and causes of death in the pediatric population.

Child maltreatment is a worldwide health problem. Previous studies have, depending on the patient population, shown significant differences in the incidence of Child Abuse and Neglect (CAN) at the emergency department. There is little evidence on the incidence of CAN in children admitted to a shockroom of a Level-1 trauma center. Children admitted to the shockroom suffer from severe injuries with a possible fatal outcome. Death resulting from CAN is the most severe outcome, which should be recognized not only to identify a potential perpetrator but also to protect siblings.

The objective of the current study was to identify the extent of non-accidental trauma in deceased Dutch children who were admitted to the shockroom of a Level-1 trauma center and to determine indicators of CAN. Medical and forensic files of all deceased children admitted between January 1, 2014, and December 31, 2018, in one of six university hospitals were retrospectively reviewed. Eligible for inclusion were children aged between 0 and 17 years admitted to the shockroom and who died due to traumatic injuries, suicide, or drowning. Excluded were children who died of natural causes. Non-accidental trauma was confirmed after evaluation of the multidisciplinary team (the Suspected Child Abuse and Neglect [SCAN] team), after forensic medical evaluation (using information of postmortem images, forensic autopsy), and/or confirmation in the courtroom. The following data was collected; age (years), gender, trauma mechanism, injuries, Injury Severity Score (ISS), medical history, family composition, and cause of death as established by the forensic physician. To determine significant differences in characteristics between cases with CAN and Accidental Trauma (AT), the Mann-Whitney U test was used for a skewed distribution, the unpaired *t*-test for a normal distribution for numerical data, and the chi-square test was used for discrete data. A factor was considered statistically significant when the *p*-value did not exceed 0.05.

In all, 137 children were included, with a median age of 5 years (IQR 1–13), and 87 (64%) were boys. CAN was confirmed in 13 (9.5%) cases, 21 cases remained undetermined and accidental trauma (AT) was confirmed in 103 cases. In young children (< 5 years of age) CAN was confirmed in a number of cases. The median age of CAN cases was 0 years (Interquartile Range [IQR] 0–2.5 years) versus a median age of 8.5 years (IQR 2–15) in the AT group (*p* < 0.001). An overview of the trauma mechanisms is shown in Table 1. In all deceased children, fractures (47/137) and Traumatic Brain Injury (TBI) (85/137) were often found, followed by injuries to the chest or abdomen due to blunt trauma (45/137). TBI was significantly associated with CAN (CAN 12/13 versus AT 64/103; *p* = 0.031). Children in the CAN group had a median ISS of 20.5 (IQR 13.75–28.75) versus a median ISS of 34 (IQR 25–50) in the AT group (*p* = 0.084). Postmortem investigations were performed in 11 CAN cases and 24 AT cases, total body Computed Tomography (CT) scan (CAN 1 vs. AT 12 children), autopsy (CAN 9 vs. AT 3 children). Additional injuries were found in 2 (18%) CAN children and 2 (8%) AT children (*p* = 0.001). In the undetermined group, 11 of 21 (52%) cases were drowning cases, 4 (19%) cases were “found dead” in their home. These cases were not evaluated by the SCAN team and did not undergo postmortem investigations other than the physical exam of the forensic specialist.

The incidence of CAN in deceased children in this cohort is 9.5%, and TBI was significantly associated with CAN. Postmortem investigations usually consist of a total body physical exam by the forensic physician. Postmortem imaging studies and a forensic autopsy are seldom reported, especially in drowning cases. Therefore, the incidence of CAN may be underestimated. Based on these findings, it is recommended that a thorough work-up in all young (<5 years of age) children be conducted to detect additional injuries, as injuries in these children are easily missed during the postmortem physical exam.

Table 1. Reported trauma mechanism.

Causes of Trauma	No. of Cases	%
Traffic Incident	38	27.7
Drowning	31	22.6
Found Dead	19	13.9
Suffocation	16	11.7
Fall from Height	8	5.8
Suicide	7	5.1
Other	7	5.1
Blunt Trauma	6	4.4
Sharp Trauma	2	1.5
Shooting Incident	2	1.5
Burn Incident	1	0.7

**Child Abuse and Neglect, Trauma, Death**

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\*Presenting Author



### E120 Scene Inspection in a Harsh High Mountain Environment: The Valle d'Aosta Pilot Program

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**Learning Overview:** After attending this presentation, attendees will be informed regarding the challenging task of performing a thorough crime scene inspection in a harsh high mountain environment such as Valle d'Aosta (VDA).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by outlining the VDA's model of cooperation between the VDA Office of Medical Examiner and the rescuers of the emergency department in order to gather proper documentation of the death scene, even in hostile conditions.

VDA is the smallest and least densely populated region of Italy, with a density of just 39 residents per square kilometer. However, the presence of 16 mountains in the region—among them are Mont Blanc, Monte Rosa, and the Matterhorn—whose peaks are higher than 4,000 meters, with nearly 200 glaciers, represents an attraction for tourists in search of high-altitude activities, such as skiing, and rock and ice climbing. Every year, nearly 800,000 tourists arrive in VDA to spend the holidays. Research and rescue interventions of missing people in natural disasters, extreme sports accidents, and motor vehicle/air crashes in such a harsh environment with suddenly changing weather conditions require well-established protocols between Armed Forces alpine rescuers, emergency department first aid teams, and hospital physicians. Forensic activities during these emergencies are not always feasible by the VDA Office of Medical Examiner, due to safety regulations, operative statutory requirements for high altitudes, the limited seating capacity of rescue helicopters, and the survivor's need for prompt response.

Since 2018, the VDA emergency department physicians certified for high-altitude first aid have attended informal training by medical examiners. During classes, the health care professionals were provided with forensic pathology knowledge and operative instructions, including forensic photography skills. A crime scene inspection form was introduced and attendees were taught proper compiling. During the second phase of the program, the first aid team was encouraged to provide feedback to the learning curve by practical experience. The feedback was evaluated and corrections have been discussed for each case with the attendees. Continuous feedback was guaranteed by on-call consultations and recurring meetings.

The results of the training course were tested in the field on January 25, 2019. An aerial collision occurred between an Italian helicopter and a French light aircraft over the Rutor glacier, at an altitude of 2,700 meters, close to the northern Italian border with France and Switzerland. The helicopter was carrying six people: the pilot, an alpine guide, and four passengers who were heli-skiing. There were three passengers on the light aircraft, all pilots who were training in mountain flying. All six passengers of the helicopter were killed in the accident; two of the three passengers of the light aircraft were found alive by rescuers. The remains of the helicopter were spread over a 400-meter area, with bodies recovered from about 50 meters from the wreckage site.

Despite the complexity of the rescue, the trained emergency team was able to accurately geolocate the seven corpses and the survivors, while collecting viable data for accident reconstruction and documenting the scene of the rescue. The bodies underwent external examination, Computed Tomography (CT) scans, and a complete autopsy at Aosta Regional Hospital. The results will be presented and discussed. The confluence of data resulting from scene inspection and from the autopsies allowed the team to reconstruct the accident as a mid-air impact during vertical take off of the helicopter and on-glacier landing of the light aircraft. The pilots of the aircraft were prosecuted for crossing into Italian territory without communicating a flight plan to Italian air traffic control. The preliminary results of the program have been discussed with authorities and a regional training program for emergency physicians is set to start in the fall of 2019. The goal of the program is also to prepare a regional protocol for scene inspection in harsh high mountain environments that could be applied in regional systems with comparable geographical characteristics.

#### High Mountain, Harsh Environment, Scene Inspection



## E121 Alternate Light Wavelength and Filter Detection of Inflicted Cutaneous Bruises

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**Learning Overview:** After attending this presentation, attendees will understand which alternate light wavelengths and filters are most likely to be effective in the detection of potential bruises.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by potentially improving the likelihood of cutaneous bruise detection during the clinical assessment of victims of violence.

Forensic clinicians often struggle with identifying and documenting bruises on victims of physical violence, particularly on those with older injuries or darker skin. The Department of Justice has recommended the use of alternate light as a tool to enhance visualization.<sup>1</sup> However, rigorous scientific evidence is lacking to broadly support the adoption of this technology for this purpose. It is also unclear how bruise age may impact the specific wavelength performance. The aim of this randomized controlled trial was to determine whether certain wavelengths of alternate light with the ultraviolet and visible spectrums are effective at improving detection of likely bruises over time.

A sample of 157 healthy adults received a bruise inflicted to the lateral deltoid using the controlled application of a paintball pellet. Quota sampling was used to ensure equal representation of six skin color categories determined by spectrophotometer. Bruises were assessed at 21 timepoints over four weeks. The order of assessment in this crossover study was randomized with assignment to alternate light (treatment) and white light (control). Alternate light bandwidths evaluated included 365nm, 415nm, 450nm, 475nm, 495nm, 515nm, and 535nm with observations made using yellow, orange, and red long-pass filters. Multilevel modeling was used to account for the correlated data structure. The multilevel structure entailed wavelength measurements nested within assessments for each subject. Marginal models were used with fixed effects to control for the following variables: skin color, gender, arm fat, age, bruise age, and observer.

The retention rate of this longitudinal study was high (95%). The sample was primarily young ( $M=24$ ,  $SD=7.6$  years) and dominantly female (73%), with an average Body Mass Index (BMI) of 26 ( $SD=6.7$ ). A total of 2,903 bruise assessments were completed resulting in more frequent bruise detection under alternate light (98.6%) compared to white light (85.7%). The odds of detection were greatest compared to white light for 415nm and 450nm using a yellow filter (OR=5.3, 95% CI: 4.3–6.5,  $p < .0001$ ; OR=4.1, 95% CI: 3.3–4.9,  $p < .0001$ , respectively), followed by the orange filter under the same wavelengths. All of the remaining bandwidths had significantly lower odds of bruise detection compared to white light. Based on these results, recommendations can be made for clinical forensic units to invest in alternate light technology to improve the detection of potential bruises.

*This research was funded by a National Institute of Justice (NIJ) Grant. Opinions, points of view expressed in this research, and products discussed represent a consensus of the authors and do not necessarily represent the official position, policies, or endorsement of the United States Department of Justice or the NIJ.*

### Reference(s):

1. U.S. Department of Justice. *A national protocol for sexual assault medical forensic examinations: Adults/adolescents*. 2<sup>nd</sup> ed. Washington, D.C.: U.S. Department of Justice, Office on Violence Against Women; 2013.

### Bruise, Alternate Light, Detection



## F1 Brazil's Forensic Science: How Could It Be Helped (and Improved) by American and European Guidelines?

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**Learning Overview:** The objective of this study is to analyze how the North American and European guidelines can help in the development of Brazil's forensic sciences. This will bring to the public the knowledge regarding the forensic sciences in Brazil, as well as bring to the public's attention the standard guidelines pointed out by the United States and Europe.

**Impact on the Forensic Science Community:** This presentation will have a significant positive impact on the forensic community, demonstrating important characteristics of forensic science that have been pointed out by the major forensic agencies worldwide. In addition, this presentation will promote a connection between what is studied in Europe and what is researched in the United States, making a parallel between the Brazilian and world forensic sciences in addition to bringing improvements to both.

In recent years, Brazil's courts have seen a dramatic increase in the use of scientific evidence. This forensic evidence often is assumed to be reliable by the courts. However, just because evidence is scientific in nature does not mean that it should not also be scrutinized before use. From the collection of the evidentiary material to the issuance of the final report, it is important that procedures be carefully established and carried out before the evidence can be presented to a fact finder. The newness of the Brazilian practice makes it ideal for pointing out ways to improve early on. To be sure, the Brazilian forensic practice has some deficiencies that must be overcome in order to have more reliable forensic evidentiary conclusions.

This study aims to evaluate two reports that were published worldwide in the field of forensic sciences and to identify how these reports could help in the development of forensic sciences in Brazil. The two reports, *Strengthening Forensic Science in the United States: A Path Forward*, and the European Network of Forensic Science Institutes (ENFSI) *Guideline for Evaluative Reporting in Forensic Science: Strengthening the Evaluation of Forensic Results Across Europe (STEOFRAE)*, were published by American and European forensic science researchers, respectively.<sup>1,2</sup> Viewed through the lens of a developing national forensic service provider, these reports demonstrate important areas of international consensus. Parallels between the two reports and Brazil's nascent practice promote border-crossing connections and elevate the field of forensic science.

The two reports each add different insight into Brazil's developing field of forensic science. *Strengthening Forensic Science in the United States: A Path Forward* provides a comprehensive view of forensic sciences and explains the general characteristics of the disciplines and how they can best be used in court.<sup>1</sup> ENFSI's report, meanwhile, focuses more on the characteristics of how to interpret scientific evidence and how to issue an expert report.<sup>2</sup> Together, they are both extremely important and relevant to the legal-forensic-criminal context.

Specific takeaways to be discussed in this study are the importance to Brazil's developing forensic practice accreditations, certifications, standards, and guidelines for quality control and the code of ethics. In 2014, two laboratories in Brazil received the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025:2005 seal, both from the National Institute of Criminalistics of the Federal Police, being the Laboratory of Forensic Genetics Expertise, and the Laboratory Expertise Service. Currently, the accreditation is being redone and both laboratories are accredited.

Another point of discussion is the usefulness of the ENFSI report in guiding Brazilian forensic scientists on the communication of scientific findings in expert reports. Among these requirements, there is balance, logic, robustness, and transparency. These are essential characteristics of a good expert report and must not be missing at the time of scientific evidence production.

Thus, both publications have much to contribute to the forensic field. Standardizing forensic practice is extremely important so that basic principles of criminal procedure are assured and, especially, that innocent people are not imprisoned based on evidence produced without minimum scientific requirements. Brazil needs to improve its forensic practices; the analysis of both reports is an important initiative for the country.

### Reference(s):

1. National Research Council (NRC), Committee on Identifying the Needs of the Forensic Science Community. (2009). *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press.
2. ENFSI (2015) *Guideline for evaluative reporting in forensic science: Strengthening the Evaluation of Forensic Results across Europe (STEOFRAE)*. At: <https://www.unil.ch/esc/files/live/sites/esc/files/Fichiers%202015/ENFSI%20Guideline%20Evaluative%20Reporting>.

### Strengthening Forensic Science, Penal Process, Scientific Evidence



## F2 Deaths During Law Enforcement Encounters

*Michael M. Baden, MD\*, New York, NY 10019*

**Learning Overview:** After attending this presentation, attendees will better understand the difficulties in identifying and investigating deaths that occur during police encounters.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the need to better determine the extent of the problem of police-encountered deaths in order to develop strategies to reduce such deaths.

Deaths during law enforcement encounters usually occur from shooting or while someone is being restrained. When someone is shot, the cause of death is usually clear and intended. The issues are whether the shooting was justified. When death occurs during physical restraint, the cause of death is not clear and the death is almost always unintended.

This presentation will address the roles excited delirium, Tasers<sup>®</sup>, drugs of abuse, acute psychotic behavior, pre-existing natural disease, and physical interference with breathing may have in causing or contributing to such deaths. The politics that are often involved in deciding what to do with such information will also be addressed.

The investigation into the death of Eric Garner illustrates many of the above issues. Mr. Garner was a 43-year-old Black male who collapsed and died in the early afternoon of July 17, 2014, while being restrained on his sidewalk by New York City Police in Staten Island. He was being arrested for selling individual cigarettes in his neighborhood. The initial confrontation, his takedown, and his death were captured on a neighbor's iPhone<sup>®</sup>. He is heard in the video saying, "I can't breathe," 11 times during the 50 seconds of struggle while pressure was placed on his neck, back, face, and nose. The video will be shown and examined.

At autopsy, Mr. Garner was 74" tall and weighed 395 pounds. There were petechial hemorrhages in his eyelids, hemorrhages in his neck muscles, and evidence of neck compression. He also had evidence of hypertensive cardiovascular disease with cardiomegaly (660 grams) and acute and chronic bronchial asthma. Toxicology was negative. The medical examiner listed the Cause of Death as "Compression of neck (choke hold), compression of chest and prone positioning during physical restraint by police. Contributory conditions: Acute and chronic bronchial asthma; obesity; and hypertensive cardiovascular disease. Manner of Death: Homicide." Other jurisdictions might have listed the cause of death in this case as excited delirium, asthma, heart disease, or acute psychosis without including the police encounter in the cause of death. Some pathologists may conclude that the death would have occurred even without police involvement. If a drug of abuse were present, it may be called a drug overdose. The manner of death may be listed as accident, natural, or even suicide if a drug is present. This makes identification of such deaths difficult.

The five-year statute of limitations relative to Mr. Garner's death expired in July of 2019 without any charge being brought after city and federal government evaluations. The ability to address this national problem of deaths during law enforcement encounters—and the community disruptions they cause—begins by knowing how frequent such deaths are, who are the victims, when they occur, and the circumstances of such deaths to be able to develop strategies to reduce them. However, no such national statistics of deaths during police encounters are kept by any agency locally or nationally. Some newspapers try to keep daily records of shooting deaths by examination of newspaper mentions and death certificates. However, there is no attempt to maintain such statistics on non-shooting deaths.

This presentation is intended to raise awareness among forensic scientists that it is necessary to address the societal calamity of deaths that occur during law enforcement encounters.

### **Custody, Excited Delirium, Restraints**



## F3 Litigating the Admissibility of Black Box Forensic Software

*Kevin Riach, JD\*, Fredrikson & Byron, PA, Minneapolis, MN 55402; Charles A. Ramsay, JD\*, Ramsay Law Firm, PLLC, Roseville, MN 55113*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the practical issues unique to *Frye* and *Daubert* litigation regarding the admissibility black box forensic software and will have learned practical strategies to deal with those issues.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the competence of lawyers and expert witnesses who face the challenge of litigating the reliability and admissibility of evidence generated by black box forensic science software.

The universe of “black box” forensic software has expanded rapidly in the past several years. From probabilistic genotyping to breath alcohol measurements to facial recognition, and more, a new wave of computer-generated evidence is becoming commonplace in criminal prosecutions. But the need for transparency in the forensic sciences and in criminal law is in tension with software developers’ desire to protect the “special sauce” behind their programs. Not surprisingly, this tension has generated new challenges for lawyers and experts. Source code review, evaluation of software testing documentation, and wrangling over disclosure of proprietary technology are increasingly critical aspects of litigating the admissibility of forensic evidence in criminal cases.

This presentation will discuss the practical difficulties with litigating the admissibility of evidence generated by black box software, including the use of protective orders and Non-Disclosure Agreements (NDAs) by software developers, the need to evaluate and challenge computer-generated evidence from not just a forensic science but also a software engineering perspective, and how non-scientists can begin to approach understanding the mechanisms employed by complex software systems sufficiently to challenge those systems in court. The presenters will share their experiences litigating the admissibility of evidence generated by probabilistic genotyping software and computerized BrAC systems, and the lessons gained from those experiences.

Attendees will learn strategies for propounding discovery to the third-party companies that develop and sell black box forensic software, as well as strategies for litigating the inevitable disputes that arise from these discovery requests. Attendees will also learn how the discipline of software engineering applies when evaluating the admissibility of black box forensic software. This presentation will address specific validation and verification standards commonly applied in the software industry and relevant to forensic software, ways to incorporate those standards into a *Frye/Daubert* proceeding, and what objections lawyers can expect from forensic software developers to the use of these standards to evaluate a software program’s reliability. This presentation will discuss resources available to lawyers to advance their understanding and obtain expert assistance with respect to these standards. Finally, attendees will gain insight into the trends in black box forensic software—what disciplines are likely to see a rise in the use of such software and how courts’ views regarding the admissibility of such software have been changing over time.

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### Black Box, Litigate, Software





## F4 The Defense Lawyer Perspective on Uncertainty in Probabilistic Genotyping

*Jessica Goldthwaite, JD\**, *The Legal Aid Society, New York, NY 10038*; *Tamara Giwa\**, *New York, NY 10038*

**Learning Overview:** After attending this presentation, attendees will better understand: (1) why the sources of uncertainty in probabilistic genotyping may mean different things to defense attorneys than to lab analysts; (2) the various sources of uncertainty that defense lawyers must incorporate into their understanding of forensic DNA case results; and (3) the importance of clear communication and transparency about the sources of uncertainty in promoting just outcomes in the criminal justice system.

**Impact on the Forensic Science Community:** This presentation will increase awareness among attorneys about the sources of uncertainty in probabilistic genotyping and encourage laboratories to adopt practices to better communicate uncertainty in their results.

In the criminal justice system, defense attorneys are always the last to know. They typically meet their clients after an investigation and arrest has occurred, and they receive the DNA casefile and reports at the end of the testing and interpretation process—once prosecutors have put together their case against the accused, and labs have examined and interpreted the forensic evidence. The defense is almost always excluded from the process of scientific interpretation and has to try to make sense of the already-completed forensic product. On the path to generating the final likelihood ratio results, laboratories may encounter various sources of uncertainty inherent in probabilistic genotyping systems. These include: (1) communicating the meaning and limitations of likelihood ratios; (2) different results among software programs; (3) unexpected occurrences in the results; (4) proposition setting and other user-defined choices in running probabilistic genotyping software; (5) the modeling of relatedness; and (6) bugs in the software. Sometimes these uncertainties are explicitly disclosed in the casefile, but sometimes they are not. Sometimes they are disclosed but buried deep in the casefile, and would escape the notice of even the most diligent attorney.

In this new scientific frontier, defense lawyers should be aware of and understand the reality of these uncertainties in order to mount a vigorous defense of the accused. Thorough investigation, case preparation, and courtroom litigation requires lawyers to first recognize the uncertainty and then decide what questions to ask. This presentation will encourage lawyers to ask some essential questions: Is uncertainty from the defense perspective the same as uncertainty from the laboratory's perspective? What are the causes and effects of uncertainty that defense attorneys must consider? What is the relationship between scientific uncertainty and the presumption of innocence?

This presentation will also advocate for greater transparency and communication by laboratories around points of uncertainty. It will encourage laboratories to ask some essential questions: What if communication with defense attorneys was frequent and open? What if error was immediately disclosed without fear? What might best practices around communicating uncertainty look like?

### Uncertainty, Probabilistic Genotyping, Communication



## F5 Bioethics and Emerging Trends in Texas for Mirandizing Juveniles

Jennifer Bennett, JD\*, 265th Judicial District Court, Dallas, TX 75207

**Learning Overview:** The goal of this presentation is for attendees to better understand how Texas has taken steps to protect the rights of juvenile suspects without creating an undue burden on law enforcement. Attendees will also have a greater understanding of emerging trends in courts that provide more protections to persons who are deemed to be in need.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by detailing the progressive measures Texas has taken to protect the rights of juvenile defendants.

The purpose of this presentation is to educate attendees about trends in juvenile interrogations within the Criminal Justice System. This presentation will help the audience understand the differences in juvenile interrogations across the United States, specifically related to confession admissibility. The progressive, more protective standard that Texas follows will be highlighted in contrast with the other jurisdictions that Mirandize juveniles similarly to adults.

Unlike most states, Texas has statutorily protected the rights of juveniles and set out requirements for law enforcement to follow in order to admit the statement of a juvenile in court. As a result, Texas law provides greater protections for juveniles than most jurisdictions, as well as more safeguards than the United States Supreme Court, which ruled most recently on issues pertaining to custodial juvenile statements in *J.D.B. v. North Carolina*.<sup>1</sup> In *J.D.B.*, the Supreme Court applied a totality of circumstances test for juvenile statement admissibility; a test which the Court decided permits consideration of a juvenile's age.<sup>2</sup>

Today, most states follow the totality of circumstances standard, which incorporates Miranda warnings and the consideration of age; however, other than implementing those requirements, each state has the discretion to apply their own standards regarding the admissibility of juvenile statements gathered by law enforcement. As the Supreme Court has yet to add a universal standard, there is a large discrepancy in how jurisdictions handle Mirandizing juveniles.

While all states have policies in place, very few are as progressive as Texas. For example, in *U.S. v. Guzman*, the Court applied a totality of circumstances test for juvenile statement admissibility, but declined to expand juvenile protections to require the presence or notification of a parent or guardian for police questioning.<sup>3</sup>

Texas is considered more conservative when handling matters of legislation, but the policies concerning Mirandizing juveniles are forward-thinking. In Family Code 51.095, the State of Texas requires juvenile suspects be given warnings by a magistrate outside the presence of law enforcement. That being said, Texas allows for the protection of the rights of the juvenile without imposing an undue burden on law enforcement.<sup>4</sup>

Furthermore, while most states follow a totality of circumstances test, it can be very subjective. West Virginia is an exception to this standard and requires juveniles in custody be immediately taken before a magistrate, circuit judge, or referee before any statements given to law enforcement be admissible in court.<sup>5</sup>

### Reference(s):

1. See generally *J.D.B. v. North Carolina*, 564 U.S. 261 (2011).
2. *Id.* at 281.
3. *United States v. Guzman*, 879 F. Supp. 2d 312, 321 (E.D.N.Y. 2012).
4. See generally *In re H.V.*, 179 S.W.3d 746, (Tex. App.—Fort Worth 2005), *aff'd in part, rev'd in part*, 252 S.W.3d 319 (Tex. 2008).
5. *State v. Rush*, 639 S.E.2d 809, 815 (2006).

### Juvenile, Protection, Statement



### F6 “I thought if I Told Them I Did It, They Would Let Me Go ...” and the Unintended Consequences of *Miller v. Alabama*

Antoinette E. Kavanaugh, PHD\*, Chicago, IL 60647

**Learning Overview:** The goal of this presentation is to educate attorneys, judges, and other criminal justice stakeholders how unreliable Miranda waivers and false/involuntary confessions create undue financial burdens on the justice system and violate an individual’s constitutional rights and the changes needed after the United States Supreme Court decision of *Miller v. Alabama*.<sup>1</sup>

**Impact on the Forensic Science Community:** Attendees will be able to describe and identify factors that contribute to youth providing unreliable Miranda waivers and false confessions and will also be able to identify unintended consequences of *Miller v. Alabama* in their respective jurisdictions.<sup>1</sup> This presentation impact the forensic science community by assisting attendees in identifying systemic changes needed in response to *Miller v. Alabama*.<sup>1</sup>

Better education among the legal stakeholders relating to the science behind juvenile Miranda waivers and juvenile involuntary confessions will lead to more reliable statements and better outcomes for juveniles and the integrity of the criminal justice system following the decision of *Miller v. Alabama*.<sup>1</sup>

Unreliable Miranda waivers and false/unreliable confessions create undue financial burdens on the justice system and violate an individual's constitutional rights. There often seems to be a disconnect between the knowledge of stake holders in the criminal justice system (law enforcement, prosecutors, defense attorneys, and judges) and the scientific research relating to vulnerabilities unique to juveniles and the need for special protections for juveniles who are being interrogated. Courts appear to frequently admit statements made by juveniles based on interrogation processes that were intended for adults and do not take into account the vulnerabilities associated with being a youth. At the conclusion of this presentation, attendees will have a better understanding of the factors that make youth vulnerable to providing unreliable waivers and confessions. By understanding the factors that make youth vulnerable, there can be a better ability to identify cases where statements made by youths are not reliable based on the findings within the scientific research. By being able to better identify improper techniques based on the scientific research and the unique vulnerabilities associated with youth, the criminal justice system can make further reforms relating to juveniles.

Regardless of the severity or actual participation in the alleged offense, many juvenile suspects believe this statement: “I thought if I told them I did it, they would let me go ....” Research findings will be presented that explain how this belief: (1) is related to vulnerabilities associated with being a youth; (2) contributes to an inability to provide a voluntary, knowing, and intelligent waiver; and (3) contributes to youths providing unreliable or false confessions.

The Criminal Justice System has started to take notice at the sentencing stage that youths are different than adults. The Supreme Court ruled in *Miller v. Alabama* that before a defendant who committed a charged crime before their 18<sup>th</sup> birthday is sentenced to Life Without Parole (LWOP), the court must consider the defendant’s: (1) home life and environment; (2) age and characteristics associated with youth; (3) the circumstances of the offense, including the defendant’s role in the offense; and (4) the possibility for rehabilitation.<sup>1</sup> While some saw this as a victory, the decision caused many unintended consequences. This presentation will discuss the following four consequences that have become especially problematic after the decision in *Miller v. Alabama*: (1) the lack of individuality of some of the responses to *Miller*; (2) the implication for the parole board systems; (3) implications for the adult prison and parole system; and (4) the procedure for obtaining a forensic mental health evaluation.<sup>1</sup>

**Reference(s):**

<sup>1</sup>. *Miller v. Alabama*, 567 U.S. 460 (2012).

**Juvenile, Miranda, *Miller v. Alabama***



### F7 “The Worst of Both Worlds”: Neurodevelopment of the Transitional Age Brain

Sandra Antoniak, MD\*, *Impavida Forensic Consulting, LLC, Fishkill, NY 12524*

**Learning Overview:** After attending this presentation, attendees will appreciate the major neurodevelopmental processes present in adolescents and young adults and its impact on their behaviors and decision-making abilities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a basic introduction to neurodevelopmental processes present in youth and young adults. Knowledge regarding the neurodevelopmental basis for behavior will enhance the legal practitioner’s ability to understand and communicate with their adolescent and young adult clients, as well as enhance advocacy efforts.

In the decades between *In Re Gault* and *Miller v. Alabama*, the legal system in the United States has sought to develop legal processes and remedies that reflect our evolving societal values while maintaining the due process rights of children and adolescents accused of major crimes.<sup>1,2</sup> In 1966, Justice Fortas famously captured the gestalt of this legal quandary when he wrote, in the majority opinion for *Kent v. United States*, that, “The child receives the worst of both worlds ... neither the protection accorded to adults nor the solicitous care and regenerative treatment postulated for children.”<sup>3</sup>

During the past two decades, the concept of the “transitional age brain,” occurring between ages 13 and 25, has become part of the child and adolescent psychological and neurodevelopmental canons. The transitional age brain, and the behaviors it produces, is precariously balanced between the surging development of subcortical regions of the brain that drive risk-taking behavior and the gradually emerging prefrontal regions that enhance behavioral restraint. Simultaneously, the novelty-seeking circuitry is primed, but the reward response is quiescent, thereby driving the adolescent or young adult to impulsively seek more extreme stimuli to feel the psychological and physical reward of the behavior (e.g., binge drinking, impulsive drug use, multiple sexual partners, sexting).

This neurodevelopmental period is also marked by significant increases in psychiatric and physical morbidity (e.g., depression, Sexually Transmitted Diseases [STDs]), high risk social behaviors such as drug and alcohol use, risky sexual practices), and mortality from violence, accidents, or suicide. To complicate matters further, intellectual disabilities, developmental delays (e.g., autism spectrum disorders), chronic medical conditions (e.g., epilepsy), trauma due to abuse and neglect, and psychosocial stressors can alter the time course, and ultimate outcome, of the individual’s neurodevelopment. These physiological or environmental effects can be exerted directly, such as occurs with brain injury, or indirectly, as mediated by hormonal systems and environmental impact on genetic expression (e.g., G x E interactions). Regardless of etiology, the above factors can impact the form and function of the transition age brain and can impact the individual’s life course.

This presentation will provide the legal practitioner with a plain language explanation of the neurodevelopmental basis of impulse-control difficulties and risk-seeking behavior that often bring adolescents and young adults before the court. Utilizing a neurodevelopmental lens will aid the legal practitioner to understand complicated issues surrounding the adolescent’s or young adult’s legal decision-making capacity, their ability to meaningfully participate in legal proceedings, and their susceptibility to peer influences and coercion.

**Reference(s):**

1. *In Re Gault*, 387 U.S. 1(1967).
2. *Miller v. Alabama*, 567 U.S. 460 (2012).
3. *Kent v. United States*, 383 U.S. 541 (1966).

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**Neurodevelopment, Adolescent, Capacity**



### F8 Is Communication Between the Surgeon and the Anesthesiologist Really Necessary? What Are the Medicolegal Implications?

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**Learning Overview:** The goal of this presentation is to evaluate the existence of bad communication between doctors in the operating room in a clinical case of professional responsibility.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees how a lack of or bad communication between an anesthesiologist and an ear surgeon prevented correct management of the clinical case, determining the fatal outcome.

**Background:** Ineffective team communication is often the cause of medical errors. This story begins with a serious error by an anesthesiologist.

**Clinical Case:** The incident, which occurred in December 2007 at the Hospital of Vibo Valentia (Calabria), is inherent in the death of a 16-year-old girl hospitalized with a peritonsillar abscess with edema.

On December 1, 2007, the girl was visited by the family doctor who diagnosed tonsillar abscess and prescribed an antibiotic and cortisone. This was followed by hospitalization after a few days. The patient was visited by the head of the Otorhinolaryngology department of the aforementioned hospital. Peritonsillar abscess with edema was diagnosed. Therapy with intravenous and cortisone cephalosporins was prescribed but did not prevent the progression of the disease. The morning after admission, the young woman was taken to the operating room for a tracheotomy, which was necessary due to the disease progression, which compromised the airways.

The anesthesiologist tried twice to give general anesthesia with curare and intubation; but without results. The myorelaxant effect of the curare caused paralysis of the respiratory muscles with consequent total occlusion of the respiratory tract. Anoxia occurred with desaturation. In this dramatic contingency, the anesthesiologist tried to perform an emergency tracheotomy, but without success. Death from cardiocirculatory arrest occurred following pharmacologically induced asphyxia. The autopsy conducted on the girl's body showed that the scalpel had also caused a lesion on the esophagus and a lesion of the large neck vessels.

At the end of the judicial procedure, the six doctors, including otolaryngologists and anesthesiologists, accused of manslaughter were sentenced.

**Conclusions:** The sentence of the Cassation of May 5, 2015, states that bad teamwork has given way to human error responsible for the death of the girl.

The Court of Cassation observes that, "Teamwork sees the institutional cooperation of different subjects, often bearers of distinct competences," placing itself perfectly in line with its own jurisprudence and of previous merit. It adds, however, that, "This activity must be integrated and coordinated, it must be removed from anarchism." For the first time in memory, the Supreme Court used this term referring to teamwork and drew the following conclusion: "For this reason the role of guide of the head of the work group is relevant," which "cannot disregard the whole activity of the other therapists, but must instead direct it, coordinate it."

Therefore, when the anesthesiologist makes choices related to his role as a medical specialist in his own specialty, he is personally responsible for the choices made. When, on the other hand, he proposes choices that belong to a context of common knowledge of other doctors, he re-emerges from the past role of head of the surgeon's first operator who, in the face of the refusal to adhere to the directives given by the anesthesiologist "well may suspend the activity," or, using the words of the judges of merit, dismiss the anesthesiologist.

In this case, regarding the specific multidisciplinary anesthetic question and in the face of decisions that interfered with the surgical choices of edema control, the Roman judges specify that it was up to the surgeon to decide the "weighting of the implications of curative anesthesia" with the consequence of "preventing anesthesia."

This was so true that he expressed his point of view, which turned out to be correct, but he did not draw the necessary consequence; that is, the duty to prevent anesthesia by possibly suspending the execution of the surgical procedure which, as was seen, was urgent but was not treated as such.

The lack of or bad communication between the anesthesiologist and ear surgeon prevented the correct management of the clinical case, determining the fatal outcome.

#### Medical Communication, Teamwork, Medical Error



## F9 Recent National Institute of Standards and Technology (NIST) Activities in Forensic Science: Examining Scientific Foundations and Innovation-to-Implementation Issues

John M. Butler, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD 20899; Robert M. Thompson, MFS\*, National Institute of Standards and Technology, Gaithersburg, MD 20899

**Learning Overview:** The goal of this presentation is to discuss recent efforts at NIST to strengthen forensic science through examining scientific foundations of methods and through studying challenges with implementing new research innovations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the importance of having documented research and validation studies to support measurement and interpretation claims in forensic science. This presentation will also review a recent innovation-to-implementation meeting held at NIST.

Involvement of the NIST in forensic science spans almost a century. Wilmer Souder, a physicist at the then National Bureau of Standards (1911–1913, 1917–1954), performed handwriting, typewriting, and ballistics analysis on more than 800 cases for dozens of Federal agencies beginning in the 1920s.<sup>1</sup> After the 2009 National Research Council Report, *Strengthening Forensic Science in the United States: A Path Forward*, NIST increased its involvement in the field. A partnership initiated in 2013 with the Department of Justice (DOJ) launched the Organization of Scientific Area Committees for Forensic Science (OSAC) and established the National Commission on Forensic Science (NCFS) as a federal advisory committee to DOJ.<sup>2,3</sup> While NCFS closed after its second two-year term in April 2017, OSAC continues with Congressional-funding and participation of more than 500 members of the forensic community.<sup>4,5</sup>

Today, NIST conducts collaborative research, partners with the community to strengthen policies and practices, convenes meetings to examine issues, and explores scientific foundations of forensic disciplines.<sup>6</sup> NIST maintains active research programs in fingerprints and other pattern evidence, including firearms and tool marks, forensic DNA, digital evidence, forensic statistics, drug analysis, and trace evidence. NIST creates forensic-specific reference materials and data sets to enable traceable measurements. NIST also funds the Center for Statistics and Applications in Forensic Evidence (CSAFE) to improve statistical support for pattern and digital evidence.

At the request of Congress, NIST has begun scientific foundation reviews of various forensic disciplines.<sup>7</sup> The first review covers DNA mixture interpretation and explores issues of relevance and reliability when testing complex mixtures involving small amounts of DNA. Additional reviews are planned to cover bitmark analysis, digital evidence, and firearms and tool marks. These reviews should increase trust in forensic methods that have a strong scientific foundation and provide strategic direction for future research in areas that need further strengthening.

In June 2019, NIST convened a Forensic Science Research Innovation-to-Implementation (RI2I) Symposium to examine challenges with moving research ideas and projects into routine use in forensic laboratories.<sup>8</sup> Presentations and discussions came from a broad range of stakeholder perspectives: academic researchers, practitioners, public and private laboratory directors, lawyers, judges, and business product managers. The United Kingdom Forensic Science Regulator and a senior project officer from the Australian National Institute of Forensic Science provided important overseas experience and perspectives. Two RI2I breakout sessions gathered additional insights from each perspective and explored implementation challenges for introducing a new technique into a toxicology laboratory.

This presentation will provide a summary of these recent activities at NIST.

### Reference(s):

1. Copies of Souder's notebooks are available in the NIST Digital Archive: <https://nistdigitalarchives.contentdm.oclc.org/digital/collection/p16009coll67/search>.
2. Butler, J.M. (2015) U.S. initiatives to strengthen forensic science & international standards in forensic DNA. *Forensic Science International: Genetics* 18: 4-20. [open access].
3. Butler, J.M. (2017) Recent activities in the United States involving the National Commission on Forensic Science and the Organization of Scientific Area Committees for Forensic Science. *Australian Journal of Forensic Sciences* 49(5): 526-540. [open access]. <https://www.justice.gov/archives/ncfs>.
4. <https://www.nist.gov/topics/organization-scientific-area-committees-forensic-science>.
5. <https://www.nist.gov/topics/forensic-science>.
6. <https://www.nist.gov/news-events/news/2018/09/nist-details-plans-reviewing-scientific-foundations-forensic-methods>.
7. <https://www.nist.gov/news-events/events/2019/06/forensic-science-research-innovation-implementation-symposium-ri2i>.

### Scientific Foundations, NIST, Implementing Research



### F10 The Elusive Holy Grail of Simple Yet Adequate DNA Mixture Evaluation Method, or How to Frame a Suspect?

Charles H. Brenner, PhD\*, DNA-VIEW, Oakland, CA 94611-1336

**Learning Overview:** After attending this presentation, attendees will appreciate that highly prosecution-biased DNA mixture analysis is still thriving and dangerous.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by alerting it the existence of an ongoing subversion of justice via misrepresentation in the court of DNA evidence, leading some suspects to be unfairly convicted based on inadequate evidence, likely including some who are factually innocent.

For 25 years, the forensic community has sought a simple but adequate method for evaluating mixtures. Some folks promoted Combined Probability of Inclusion—the idea of distilling the DNA data down to an unfortunately elusive concept of “inclusion” or not—as filling the bill. For several reasons, it was never good; finally the general understanding of the necessity of dealing with dropout turned the tide against it. The essence of “dealing with dropout” is being able to calculate the probability of some suspect allele to be missing from the mixture data even while hypothesizing that the suspect did contribute.

At present, the general consensus is to confront dropout head-on with a “continuous” approach modeling the phenomenon of peak height stochastic uncertainty. This presentation argues that a continuous model can be simple and elegant in concept, but recognizes that it is tedious to compute. It needs a computer. Hence the less comprehensive “semi-continuous” approach has its adherents and perhaps provides a useful compromise dropout capability.

At a recent murder trial, a Dr. X, expert witness for the prosecution, presented a surprising and unfamiliar mixture calculation method that purports to be the long-sought holy grail of adequate but simple. Dr. X deems a mixture locus to be “informative” if all expected alleles, especially those of the suspect, are observed. Simple binary Likelihood Ratios (LRs) are computed and accumulated across the “informative” loci. The remaining loci are discarded (conveniently evading the difficulty of a dropout calculation); Dr. X testified that omitting them is to omit further evidence against the suspect, hence omitting them favors the suspect.

That is, of course, well-known to be too good to be true. The truth is the opposite. The so-called “non-informative” loci are where the strong exculpatory evidence for an innocent suspect comes from. (Not seeing a suspect’s bootprints on the burgled property is evidence he wasn’t there.) Omitting them is cherry-picking that frames the suspect. Dr. X’s computation will *never* produce a suspect-benefitting  $LR < 1$ . It can only deliver a number that, to a small or large extent, hurts any suspect.

This is notable because, per Dr. X, Dr. X has testified upward of 1,000 times. If 50 out of each 100 cases rested significantly on the DNA evidence and had less than 100% of the suspect’s profile recorded in the mixture, then those 50 cases need re-evaluation. A handful will be actually innocent. It is likely that numerous innocent people are in prison because courts were gulled by this enticing but anti-mathematical method

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#### DNA, Injustice, Frame



## F11 DNA Confronts Bayes and There's Trouble

Charles H. Brenner, PhD\*, DNA-VIEW, Oakland, CA 94611-1336

**Learning Overview:** After attending this presentation, attendees will have learned that mathematics applied to the real world has its limitations, which we are beginning to come up against with some of the advanced problems that recent DNA methods try to deal with.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting humility, modesty, and some disappointment.

DNA evidence enters legal decision-making via a Bayesian framework. The framework has two components: an objective calculation of the evidentiary strength of the DNA, and a step in which the decision-maker (the court, judge, or jury) incorporates that information to sway their subjective opinion. Whether the swaying occurs according to Bayes Theorem, or whether that's a convenient fiction and a vain hope, is irrelevant to the present discussion. The only relevant point here is that the mathematical calculation is an objective process. Everything subjective or otherwise outside the knowledge of the DNA analyst is relegated to the domain of the court where it sensibly belongs.

This framework has served well historically. The evidential value of DNA data is a Likelihood Ratio (LR) computed by mathematical calculation based on a model. "Model" means a mathematical model of reality—a quantitative theory as simplified as reasonable but as complicated as necessary—that describes how the DNA data comes about. A model that deals only with measurements—allele sizes, allele population frequencies—lies within the DNA analyst's proper purview.

A dilemma arises when the DNA analysis model requires a prior probability assumption about something that can't be thought of probabilistically, or which current scientific knowledge cannot provide.

Two examples arise mostly from advanced (i.e., continuous model) mixture analysis. The analysis requires assumptions about the number of contributors, and there is no mathematically sound way to choose. Deciding how to weigh among different numbers of contributors requires assuming for example that a three-person mixture is *à priori* (i.e., without even looking) more probable than a four-person, but it is hard to imagine data that allows such an inference. All one can do is hope that the choice does not matter much. Another vexing problem arises when adjusting a "degradation" parameter in the model in order that the calculation be correct, assuming degraded contribution from the suspect. If that substantially increases the LR, one has created a more satisfying explanation of the mixture—but that end doesn't justify the means. Even if the suspect happens to be connected to the mixture, that doesn't justify framing him to prove it.

Parentage attribution can be problematic. Suppose a child shares alleles with an alleged father at all but two or three Short Tandem Repeat (STR) loci. Three hypotheses stand out: (1)  $H_p$ =man is the father and there are a few mutations; (2)  $H_a$ =man is the uncle ("a" for "avuncular"); or (3)  $H_0$ =man is unrelated to the child.

When  $H_a$  isn't under consideration, a reasonable DNA analysis reports the LR supporting  $H_p$  over  $H_0$ , properly leaving the question of the prior probabilities for  $H_p$  versus  $H_0$  to the court. With two different versions of non-paternity— $H_a$  and  $H_0$ —similar could be done only by making an assumption as to the relative prior probabilities of  $H_a$  versus  $H_0$ , but, at least for the DNA laboratory, that is not possible.

Further examples can be listed.

The conclusion from these problematic situations is not that we need better mathematics; the traditional Bayesian framework is the principled mathematical approach. Some mathematical-seeming problems simply don't have a clean solution.

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### Bayes, DNA Evidence, Limitations





### F12 Probabilistic Genotyping in the Courtroom: Admissibility, Families, Secondary Transfer, and Competing Statistics

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**Learning Overview:** The goal of this presentation is to convey the difference between different statistics, specifically Random Match Probabilities (RMPs) and likelihood ratios for the purpose of expert witness testimony, the importance and nuances of admissibility hearings for probabilistic genotyping, and the use of the familial diagnostic in casework and the courtroom.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how probabilistic genotyping is changing the way the forensic DNA expert testifies in the courtroom, as well as the way counsel presents their case. It is important for attorneys and scientists to be provided with the background information on applications and limitations of expert witness testimony on probabilistic genotyping.

STRmix™ went live for casework in the first United States laboratory in 2014. Since 2014, more than 40 additional laboratories have also gone live for casework with many additional United States laboratories in various stages of validation and implementation.<sup>1</sup> This presentation will discuss the differences between the RMP versus the Likelihood Ratio (LR) and how transitioning to LRs changes the way DNA evidence must be offered in court. The initial difficulties facing forensic DNA experts and attorneys working in the United States court system were primarily regarding admissibility and how to explain this new technology to a jury. The admissibility of probabilistic genotyping in the courtroom is currently making its way throughout all levels of the legal system. A discussion on filing motions *in limine*, in addition to responses to these motions, from jurisdictions with and without previous admissibility rulings will be addressed.

Nearly five years later, new challenges are emerging regarding testimony on probabilistic genotyping. What is the difference between different probabilistic systems? Is this case covered by this past admissibility hearing? What information can be garnered from these types of analyses other than a statistic? Can this analysis provide any support for or against a secondary transfer argument? What does this mean for relatives of the victim and/or person of interest? The familial diagnostic has been employed at DNA Labs International (DLI). This tool is utilized by DNA analysts to help evaluate probabilistic genotyping results and determine when reference standards from primary relatives may be requested. The effects of the use of this diagnostic in the courtroom will be examined.

Probabilistic genotyping has evolved how forensic DNA evidence is presented to the jury from the forensic DNA expert and the attorneys. Training, care, and consideration must be applied for the prosecution and defense attorneys and the respective DNA experts. There are probative applications to this testimony as well as limitations. As always, it is important to not overstate or understate the evidence at hand. Professional responsibility on presenting probabilistic genotyping evidence is paramount with the implementation of any new technology.

#### Reference(s):

<sup>1</sup>. *Labs Live*. STRMIX, accessed July 31, 2019, <https://johnbuckleton.files.wordpress.com/2019/07/labs-live.pdf>.

#### Probabilistic Genotyping, Likelihood Ratio, Expert Testimony



### F13 Scientific Transparency vs. Trade Secret: Issues Surrounding Disclosure of Computer Software Programs, User Manuals, and Source Codes

Jennifer Friedman, JD\*, Los Angeles, CA 90068

**Learning Overview:** The goal of this presentation is to provide an opportunity for a robust discussion about the legal and practical issues surrounding access to proprietary software programs, user manuals, and source codes of programs used in criminal prosecutions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing attendees' knowledge of the law on trade secrets and the relationship between the trade secret privilege and a defendant's constitutional rights, specifically as they relate to access probabilistic genotyping software programs, user manuals, and source codes.

**Proposition:** There is a tension between a defendant's Constitutional right to due process and confrontation and a software developer's proprietary interest in his software that has not yet been resolved by the courts.

The use of computer software programs in criminal prosecutions and, in particular, in the interpretation of DNA evidence has become ubiquitous. Indeed, the majority of laboratories in this country now use some type of probabilistic genotyping program for interpreting DNA results. DNA is not the only area in which computer software programs are used to prosecute, and in some cases exculpate, suspects. Computer software is used to help determine blood alcohol concentrations, risk assessments, and facial recognition, among other issues. Some probabilistic genotyping programs are open source, meaning scientists may review the software and source code and learn how the program operates. However, the two most widely used probabilistic genotyping programs are largely unavailable for review because their developers claim a proprietary interest. Access to their source codes has been thwarted by claims of trade secrets made by developers of the computer software program and arguments that access is unnecessary. While trial courts have generally accepted such claims, there remain no published Court of Appeals rulings specifically addressing the issue of whether upon a showing of necessity, a criminal defendant should have unfettered access to a computer program, its source code, and its user manual when results obtained from the program are being used to prosecute him.

In *People v. Superior Court (Dominquez)*, a California Court of Appeals held that the trial court had erroneously granted the defendant's motion for access to the computer program, the user manual, and the source code for the STRmix™ program and sent the case back to the trial court for further proceedings. Presumably, the defendant will attempt to make a stronger showing as to why the computer software program, source code, and user manual are necessary, and why access should be ordered. The computer software developer will have an opportunity to explain why he believes the trade secret privilege trumps the defendant's right to access.

A different California Court of Appeals, in an unpublished opinion, acknowledged the importance of this issue, but nevertheless failed to address it. "This court recognizes the important disputes underlying appellant's evidence-based positions and the amicus briefing submitted. Clarifying the circumstances necessary to trigger production of source code related to mathematically complicated and machine-derived DNA evidence and the scope of testimony from experts discussing such methodology when used to support a conviction is a matter of great concern in future cases."

While this presentation will be presented from the perspective of a defense attorney, the goal is to facilitate a discussion between prosecutors, judges, and criminalists about the role transparency plays in forensic science, the importance or lack of importance of gaining open access to the underpinnings of computer software used in criminal prosecutions, as well as the legal and constitutional implications of granting or denying disclosure.

The focus of this discussion will on probabilistic genotyping programs, but this discussion will have implications for other types of computer software and source codes used in breathalyzers, risk assessment tools, facial recognition programs, and programs used by the government to track computer use.

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#### Probabilistic Genotyping, Source Code, Access



### F14 The Fast and the Furious

*Matthew J. Marvin, BS\*, Ron Smith and Associates, Inc, Collinsville, MS 39325; Sarah Chu, MS\*, Innocence Project, New York, NY 10013*

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**Learning Overview:** After attending this presentation, attendees will be aware of the risks and costs of the limited examination procedures that are currently being implemented to address crime laboratory backlogs.

**Impact on the Forensic Science Community:** Limited examination procedures carry risks and costs and have considerable criminal justice impacts. This presentation will impact the forensic science community by describing each type of procedure, articulate the costs and risks, and provide recommendations for moving forward.

The 2014 Census of Public Funded Forensic Crime Laboratories estimated that public forensic science service providers possessed a backlog of 570,100 requests. In 2014, 63 public forensic science service providers conducted latent print functions for a total of 67% of federal, 53% of state, 62% of county, and 88% of municipal jurisdictions. Backlogs of latent print requests grew from 6% in 2009 to 12% of latent print requests in 2014. Consequently, public forensic science service providers have been seeking ways to increase casework efficiency and reduce their backlogs. A myriad of different solutions to address latent print backlogs have been implemented across the United States. Limited examinations, eliminating verifications, set rules on exclusions, and incomplete searches for prints are just a few of the methods that have been proposed and implemented. While these procedures may be effective means to reduce backlogs, they may also produce a different set of risks and costs, and should not be considered as a best practice.

This presentation poses the question: What is the cost of these policies? This presentation will include case examples of missed evidence due to incomplete or rushed examinations, with an emphasis on the long-term impact of shortcuts in friction ridge examination. Consequences include the collateral consequences of pre-trial detention, not collecting or not analyzing relevant evidence in a case, reducing the latent print examiner's exposure to complex cases, or having no record of evidence that may turn out to be important in a case post-conviction. Although identifications and inclusions are typically the focus of latent print examinations, ensuring that exclusions are properly called is also critical to identifying the person who committed the crime and freeing the innocent.

This report submits that when evidence is not processed and analyzed in full, the forensic scientists cannot tell the whole story the evidence can tell. Additionally, quality assurance methods, such as increased documentation, are not a panacea when evidence has not been collected or analyzed. Ultimately, every one of these strategies carries risks and criminal justice costs and this presentation declares that the best practice is to collect, document, and analyze all prints at a crime scene. Finally, the question is posed: "What is the role of a forensic science laboratory in the criminal justice system?" as a guiding question for forensic science service providers as they contemplate whether they will engage in backlog reduction strategies. This presentation will conclude with recommended approaches or policies to address the potential risks of limited examination and other backlog reductions strategies if they must be applied.

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#### **Latent Print, Risk Management, Criminal Justice Outcomes**



### F15 The Nose Knows? Residual Odor and Cadaver Dogs: A Review

*Dana Delger, JD\*, The Innocence Project, New York, NY 10013*

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**Learning Overview:** After attending this presentation, attendees will understand some of the scientific problems faced when using dogs to detect places where human remains once were and the current state of the law regarding the admissibility of dogs for this purpose.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining the current state of the science involving cadaver dogs, particularly as it relates to so-called “residual odor,” and how that science has been understood, or misunderstood, by courts. This presentation will provide an overview for the historical uses for cadaver dogs and the trajectory toward their current use to detect residual odor. Specifically, the presentation will discuss the science (and lack thereof) supporting these determinations and other problems with using dogs in this manner, including cognitive bias and prejudice.

Dogs have long been used by human beings for their superior scenting abilities, with reports of dogs finding missing or absconded people going back to medieval times. Dogs, as natural scavengers, also have a long history of being used to find deceased human remains. In 1809 Bavaria, a court clerk used his own dog to ostensibly find the victims of the “Bavarian Ripper,” and in 1974, the first purposely trained dog—a Golden Retriever named Pearl—recovered the remains of a deceased college student in upstate New York.

This use, and the use of dogs to find the extant remains of other deceased persons (such as after a natural disaster), is relatively non-controversial; remains are either found or they are not. However, there has been a rise in the use of dogs to ostensibly detect places not where human remains *are*, but where human remains *have been*—days, weeks, months, or even years after those remains have been removed. There is, however, little empirical evidence that dogs are capable of such feats. There is also no evidence about how long such odors could last, and how that may vary with differing environmental conditions. Nor is it understood what, in particular, dogs are detecting when they alert to human remains, and how that may differ when those remains are no longer present. The extremely limited research in this area will be discussed. This presentation will also cover how cognitive bias may play an outsize role in these cases, given the evolutionary relationship between humans and dogs. Scientific research showing how jurors credit and understand dog evidence will also be examined.

Despite these issues, the use of this evidence is on the rise, with at least a half-a-dozen active cases at various stages of litigation in the first half of 2019 alone. Yet courts have failed to fully grapple with these and other problems with using dogs in this manner; many are refusing to subject the claimed abilities of the dogs to the scrutiny demanded of scientific evidence. These decisions and the law underlying the admissibility of this evidence will be examined.

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#### Cadaver Dogs, Admissibility, Residual Odor



### F16 The Evolution of Wrongful Convictions in Fire Cases

Terry-Dawn Hewitt, LLM\*, McKenna Hewitt, Denver, CO 80224-3703; Wayne J. McKenna, LLB, McKenna Hewitt, Denver, CO 80224-3703

**Learning Overview:** After attending this presentation, attendees will have learned of the growing problem of wrongful convictions in fire cases resulting from faulty fire science. Attendees will learn to recognize the issues that most often cause wrongful convictions in fire crime cases and how these issues are evolving, in part due to steps being taken to implement the National Academy of Sciences/National Research Council (NAS/NRC) Report.<sup>1</sup> Recognizing these issues is the critical first step to avoiding the pitfalls that lead to wrongful convictions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a wake-up call to fire investigators, prosecutors, defense attorneys, and others involved with criminal fire cases. This wake-up call comes as people become sensitized to the common issues running through wrongful conviction cases that arise from misapplied fire science. From examining these common issues, those involved in prosecuting, defending, or testifying in a fire crime case can learn lessons that reduce the likelihood of being involved in a wrongful conviction.

Post-conviction relief in fire crime cases began slowly to emerge in the 1990s, largely coinciding with early editions of National Fire Protection Association (NFPA) 921 *Guide to Fire and Explosion Investigations*, which publicized misconceptions in commonly held beliefs about fire behavior and fire patterns that were simply wrong.<sup>2</sup> Over time, NFPA 921 and its companion, NFPA 1033 *Standard for Professional Qualifications for Fire Investigator*, have together raised the bar for fire investigations by incorporating information about reliable fire investigation methodology along with developments in the knowledge base of fire science.<sup>3</sup> These two documents have long ago become entrenched in the fire investigation community. They form the basis of the two international fire investigator certification programs, play a significant role in fire investigator training, and have become widely accepted as foundational works for fire investigators. NFPA 921 has also become a favorite tool in *Daubert* challenges of fire investigation experts across America.

One would think that by now, almost 30 years after NFPA 921 was first published, cases of post-conviction relief and exonerations based on faulty fire science would be abating, at least after cases from the pre-NFPA 921 days were adjudicated to finality. Not so. Post-conviction relief for fire crime cases resulting from misleading or bad fire science continue to grow. This presentation will identify the common issues that arise from examining the court decisions in such cases. The way in which the issues in post-conviction relief or *habeas corpus* applications are evolving in response to initiatives flowing from the NAS/NRC Report will also be explored. Finally, the impact on wrongful conviction cases of the two industry standards governing fire investigations, NFPA 921 and NFPA 1033, will be analyzed.

#### Reference(s):

1. National Research Council (NRC), Committee on Identifying the Needs of the Forensic Science Community. (2009). *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press.
2. Tech. Committee on Fire Investigations. *NFPA 921 Guide for Fire and Explosion Investigations*. (Quincy, MA: Natl Fire Protection Assn, 1992 to 2017.)
3. Tech. Committee on Fire Investigator Professional Qualifications. *NFPA 1033 Standard for Professional Qualifications for Fire Investigator*. (Quincy, MA: Natl Fire Protection Assn, 2003 to 2014.)

#### Fire Investigation, Wrongful Convictions, Consensus Standards



### **F17 Post-Conviction DNA Testing: A Law School and University Collaboration as a Model to Identify and Evaluate Post-Conviction Cases**

*Katherine A. Roberts, PhD\**, Hertzberg-Davis Forensic Science Center, Los Angeles, CA 90032-4210; *Paula Mitchell, JD*, Loyola Law School, Los Angeles, CA 90015; *Cassandra Olsen, JD*, Loyola Law School, Los Angeles, CA 90015; *Nikki Herst-Cook, JD*, Loyola Law School, Los Angeles, CA 90015; *Mehul B. Anjaria, MS, MBA DNA Consulting LLC*, Los Angeles, CA 90071

**Learning Overview:** After attending this presentation, attendees will understand the California Forensic Science Institute-Loyola Project for the Innocent (CFSI-LPI) case review model, with particular emphasis on case prioritization, filing, and arguing motions requesting DNA testing, and strategic approaches to case resolution.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a Law School-University collaboration model to tackle the logistical challenges related to post-conviction DNA testing.

The Postconviction DNA Testing Law (Senate Bill 1342) was passed in California in 2001. This law provides a mechanism for inmates to seek post-conviction DNA testing of evidence to ensure those wrongly convicted are able to prove their innocence through the use of newly developed technology. SB 1342 states that in felony conviction cases, government agencies must retain evidence for potential post-conviction DNA testing. With respect to retention of biological evidence, SB 1342 specifies agencies should retain all items that have a “reasonable likelihood” of containing biological evidence. However, it is virtually impossible for persons seeking to exonerate themselves through post-conviction DNA testing to meet the requirements of California’s statutory scheme without some legal and expert assistance. Under California’s DNA testing statute (Penal Code §1405), a request for DNA testing must comprise several criteria. These include a claim of innocence; the details regarding every reasonable attempt made to identify both the evidence to be tested; and, the specific type of DNA testing sought. Further, the statute requires an explanation as to how, in light of all the evidence, the requested DNA testing would raise a reasonable probability that the convicted person’s verdict or sentence would be more favorable if the results of DNA testing had been available at the time of conviction.

In 2019, the CFSI within the School of Criminal Justice and Criminalistics at California State University, Los Angeles, established a partnership with Loyola Law School’s LPI in order to review cases and locate and test evidence related to violent felony offenses where actual innocence may be demonstrated. The CFSI-LPI team was awarded Department of Justice (DOJ) funding under the 2019 Postconviction Testing of DNA Evidence solicitation to provide critical assistance to individuals convicted of a serious felony that meet specific criteria. Collectively, the CFSI-LPI team reviews violent felony cases to locate biological evidence for submission to a forensic laboratory for DNA testing to demonstrate their innocence or assist in their exoneration.

This presentation will provide an overview of the CFSI-LPI case review model, with particular emphasis on the identification and evaluation of cases that meet the criteria under CA Penal Code §1405 for post-conviction DNA testing. This presentation will also include a discussion of the prevalence of skewed eyewitness testimony, false or misleading forensic evidence, inadequate crime scene investigations, and false confessions among cases under review by LPI. Further, there will be debate that these long-standing issues in criminal investigation may be exacerbated when coupled with the pervasive, if unintentional, bias that recent literature shows may alter an investigator’s perception of the evidence. This presentation will also describe obstacles to this project design during the first year of the grant award and will demonstrate how the CFSI-LPI model provides an invaluable forensic science experiential learning experience where graduate students participate in a service-learning social justice project. Exposing forensic science graduate students to actual innocence cases provides them with a clearer understanding of how the justice system can sometimes make mistakes that lead to dire consequences.

#### **Post-Conviction, DNA Testing, Law School-University Collaboration**



### F18 An Evaluation of Sample Preparation Techniques for Cannabis and Cannabis Products

*Kelsey Cagle\**, State College, PA 16801; *Jessica Westland*, MPS, Agilent Technologies, Inc, Wilmington, DE 19808; *Frank Dorman*, PhD, University Park, PA 16802-4500

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**Learning Overview:** This presentation will discuss the results from a consolidated sample preparation technique for cannabis and cannabis products that will allow for the analysis of incurred pesticides residues and potency evaluations, ideally from the same sample preparation process. Data from both Gas Chromatography/Tandem Mass Spectrometry (GC/MS/MS) and High-Performance Liquid Chromatography (HPLC) will be shown to demonstrate the effectiveness of this approach. Furthermore, the cannabinoid profiles for the samples will be determined and the incurred pesticides residues will be measured for each sample as a result of proper sample preparation methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a more comprehensive overview of sample preparation techniques that can be used for a host of analyses using a complicated matrix like cannabis or cannabis product that will demonstrate to users the effectiveness of the sample preparation techniques but also the ease the expense of the technique.

Sample preparation is an essential part of method development that is critical to successful analytical measurements. In short, the goal for organics analysis is to reduce and simplify the complex sample matrices to a form that is applicable for instrumental analysis without bias to potential target and desired non-target compounds. With cannabis and cannabis products, however, the analyst is faced with a very challenging matrix and targets that may range from trace level through percent level, thus placing considerable demands on the sample preparation techniques. The most effective sample preparation techniques will allow for a precise and accurate analytical measurement of both cannabinoids and incurred pesticides present within the samples. Cannabinoid profiling has been utilized in the field for some time now due to the legal aspects associated with cannabis and cannabis products; however, incurred pesticide recovery analysis is currently a hot-button topic and growing in popularity. The need to perform pesticide analyses on cannabis and cannabis products is rising because of safety concerns for the users and growers that are present due to the lack of regulations in place in regard to pesticides and the cultivation of cannabis plants. Cannabinoid profiling alongside incurred pesticide analyses could also be useful to law enforcement in order to track where the cannabis and cannabis products came from, including dealer and grower, if any patterns were to be detected. Those patterns could be of importance to law enforcement when trying to investigate cannabis cases.

Recently, sample preparation for cannabis and cannabis products has moved toward Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS)-like procedures due to their high throughput and lower costs. QuEChERS-like procedures provide a more generic approach that is well suited for a variety of products and also provides sample cleanup that increases compatibility for both GC and LC separations. QuEChERS' appeal to cannabis work is its ability to be modified in order to deal with matrix effects commonly associated with cannabis.

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#### Cannabis, HPLC, GC/MS

## F19 How Should Uncertainty Be Expressed and Communicated?

Veronica Scotti, LL.M.\*, Politecnico Di Milano, DEIB, Milano 20133, ITALY; Alessandro M. Ferrero, M.Sc., Politecnico di Milano, DEIB, Milano 20133, ITALY

**Learning Overview:** After attending this presentation, attendees will better understand the role played by forensic metrology in providing a universal method for expressing and communicating uncertainty. The method, originally thought for expressing uncertainty in measurement, can be applied to express and communicate uncertainty related to scientific theories and data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a better awareness that uncertainty can be expressed and communicated in a universal way, independently of the mathematical tool used to evaluate it. The methods of metrology can be usefully applied to express and communicate uncertainty also when non-physical quantities are considered, such as expert testimonies.

An important outcome of the 2009 National Academy of Sciences (NAS) and 2016 President's Council of Advisors on Science and Technology (PCAST) Reports is a greater awareness that any scientific method is inherently uncertain, and the provided results are consequently affected by uncertainty that must be expressed and communicated to avoid presenting incomplete or even misleading evidence to the trier of fact.<sup>1,2</sup>

These documents have opened the discussion not only on how to assess the scientific validity of forensic science methods in terms of uncertainty, but also on how to communicate it.<sup>3,4</sup> In particular, the discussion focused on who is communicating, what is being communicated, in what form is uncertainty communicated, to whom it is communicated, and to what effect.<sup>4</sup>

While some of these points involve psychological issues that must be considered, especially when communicating uncertainty to individuals and communities without a proper technical background to avoid undesired negative perceptions, other points, namely what and in what form is communicated, involve technical issues.

In this respect, the fundamental concepts of metrology, as defined by the International Vocabulary of Metrology (VIM) and the Guide to the Expression of Uncertainty in Measurement (GUM), provide a helpful background to communicate uncertainty in a scientifically sound way, encompassed by the international Standards.<sup>5,6</sup>

It is worth quoting clause 0.3 in the Introduction section of the GUM: “*just as the nearly universal use of the International System of Units (SI) has brought coherence to all scientific and technological measurements, a worldwide consensus on the evaluation and expression of uncertainty in measurement would permit the significance of a vast spectrum of measurement results in science, engineering, commerce, industry, and regulation to be readily understood and properly interpreted.*”<sup>6</sup>

Moreover, the proposed method is *universal*, that is: “*the method should be applicable to all kinds of measurements and to all types of input data used in measurements*” (clause 0.4).<sup>6</sup>

This presentation seeks to show that the uncertainty definition proposed by the GUM is indeed universal and can be usefully employed in evaluating, expressing, and communicating uncertainty also when non-physical quantities have to be measured (the so-called *soft measurements*).<sup>6</sup>

It will be shown that the method for quantitatively evaluating uncertainty is presently based on the mathematical theory of probability. It will be shown as well that this mathematical approach has some limitations, due to the required mathematical assumptions on the way limited information is modeled, and that more recent mathematical theories are available, such as the theory of evidence, to model limited information in a more general way than probability, thus leading to the ability to also express, evaluate, and communicate uncertainty on non-physical quantities, such as human reasoning, in a strict and scientifically sound way.

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1. National Research Council (NRC), Committee on Identifying the Needs of the Forensic Science Community. (2009). *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press.
2. President's Council of Advisors on Science and Technology (PCAST). *Report to the President: Forensic Science in Criminal Courts: Ensuring Scientific Validity Of Feature-Comparison Methods*. (2016). Washington, DC: Executive Office of the President of the United States.
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4. van der Bles Anne Marthe, van der Linden Sander, Freeman Alexandra L.J., Mitchell James, Galvao Ana B., Zaval Lisa, Spiegelhalter David J. Communicating uncertainty about facts, numbers and science. *R. Soc. Open Sci.* 6: 181870 (2019).
5. *International vocabulary of metrology: basic and general concepts and associated terms (VIM)* (Geneva: ISO, 2007).
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### Methods, Data, Uncertainty





## F20 Breath—A Bodily Fluid: Semantics, Syntax, Syllogisms, Euphemisms, and Science

*Dennis C. Hilliard, MS\*, RI State Crime Laboratory, Kingston, RI 02881-1966; Gil Sapir, JD, Chicago, IL 60680*

**Learning Overview:** After attending this presentation, attendees will understand how a novel definition of breath as a bodily fluid affects the applicability of breath alcohol testing in the workplace.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by discussing practical aspects of breath sample testing in non-regulated workplace testing as a gas versus a bodily fluid.

Employers have developed drug-free workplace policies. Nationally, 46 states have laws imposing drug-testing restrictions specifying testing methodologies, use of test results, and privacy issues.<sup>1</sup>

Statutory workplace testing of controlled substances permit urine, blood, tissues, or other bodily fluids and may include alcohol. Analysis is by a federally certified laboratory (common body fluids are: saliva [oral fluid], vitreous humor, cerebral spinal fluid, synovial fluid, urine, blood, bile, semen, etc.). Frequently, blood is initially analyzed using the Enzyme-Multiplied Immunoassay Technique (EMIT) and Ultraviolet (UV) analysis. Positive test results require confirmation testing employing Gas Chromatography/Mass Spectrometry (GC/MS) or technology recognized as being at least as scientifically accurate (e.g., Liquid Chromatography/Mass Spectrometry [LC/MS] or UV/Infrared [UV/IR] spectroscopy). Labor statutes usually entail “controlled substances” and may include alcohol.<sup>2</sup>

Driving Under the Influence (DUI) statutes mandate blood, urine, or breath for drug and alcohol (ethanol and low molecular-weight alcohols) testing. Breath alcohol testing is conducted with a preliminary or evidential testing device using expired alveolar air.<sup>3</sup> Breath alcohol testing devices do not directly test for alcohol in blood. If blood or any liquid is placed into the unit, it will malfunction.

Breath alcohol testing devices are not considered laboratory clinical-grade instrumentation and are not commensurate with GC/MS’s accuracy and reliability for determination of blood alcohol concentration. If a blood test for alcohol determination is required, then blood is drawn (serum, plasma, whole) and analyzed with GC/MS head space analysis procedures.

Statutes are constructed for clarity. Testing for drugs and alcohol is inconsistent. In many states, breath alcohol testing in non-regulated workplaces statutes is not mentioned. Urine testing for alcohol is allowed for easy sample collection without any question of accuracy. States need to change arcane statutory language with an understanding of scientific nuances necessary to clearly define appropriate terminology with its intended implementation. Otherwise confusion and imaginative nonsense exists.

A creative interpretation for workplace alcohol intoxication testing, absent designated statutory or contractual language, seeks to define breath as a “bodily fluid” for statutory testing compliance.

Matter exists as a solid, liquid, or gas. A liquid describes a state of matter, whereas a fluid is any substance that flows. Liquid is a substance that flows in a natural state, flowing neither solid nor gaseous. A fluid is a liquid or gas, composed of elements or particles that freely change their relative positions without separating.<sup>4</sup>

Blood, with oxygen, carbon dioxide, nitrogen, and alcohol, is a liquid fluid. However, components in the blood are actively exchanged between the blood and the air in the lungs and they become a “gas” component within the breath.

Breath is a derivative factor of blood. The air people breathe is a gas. It is compartmentalized in the lungs for an exchange of oxygen and carbon dioxide, which is essential for life. The breath/gas expired from the respiratory system should be considered a bodily fluid—a technical distinction due to practical methods of measurement. However, common sense is paramount. Bodily liquids cannot replace breath and breath cannot replace bodily liquids in living organisms. Creatively contrived attempts to circumvent and redefine basic fundamental science are specious and problematic. If legislators or employers want to employ breath alcohol intoxication for termination under a drug-free workplace policy, then labor statutes or contracts should be amended specifying the use of breath as a testing specimen (consult the statute’s history and committee comments for legislative intent).

Semantics, syntax, syllogism, and euphemisms are irrelevant to breath alcohol as a bodily fluid. Common sense should prevail—statutory construction and the novel interpretation of breath alcohol analysis for workplace termination is at issue, not the science.

### Reference(s):

1. *United States Department of Labor’s Drug-Free Workplace Act of 1988, 41 U.S.C. 81.*
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3. *Conforming Products List of Evidential Breath Alcohol Measurement Devices, 82 FR no. 211, p.50940, Nov. 2, 2017.*
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### Breath Alcohol, Bodily Fluid, Workplace



## F21 Secure Continuous Remote Alcohol Monitor (SCRAM): Judicial Liaison As Expert Witness Controverted

Donald J. Ramsell, JD\*, Wheaton, IL 60187; Gil Sapir, JD, Chicago, IL 60680; Raul Ayala, JD, Los Angeles, CA 90012

**Learning Overview:** After attending this presentation, attendees will appreciate the necessity of questioning credentials, qualifications, and competency of SCRAM's crafted expert witness.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through recognition and understanding of an expert witness pursuant to Federal Rules of Evidence and *Daubert* criteria.<sup>2,3</sup>

SCRAM has problems with its product, marketing, and usage, which appear compounded through its expert witnesses.<sup>1</sup> The expert witness' existence is created and perpetuated by the legal system. But for the Rules of Evidence, consulting and testimonial evidence would not exist. Expert witnesses are derived from five general categories of expertise: lay people; technician/examiner; practitioner; specialist; and scientist based upon their knowledge, education, skill, practical experience, and training.<sup>4</sup> They are hired in anticipation of litigation. Experts have an ethical duty of candor and full disclosure. Omissions and misrepresentations may be considered ethical violations. Judges control the determination of good science, evidential reliability, expert witness qualifications, and competency under *Daubert*.<sup>3</sup> The expert may be qualified but not competent to testify and offer their opinion. However, as litigation support professionals, they are responsible for the losses they cause to foreseeable plaintiffs, including violations of a person's civil rights.<sup>5-9</sup>

SCRAM is designed to measure alcohol content while it diffuses through a person's skin as insensible perspiration.<sup>10</sup> It is manufactured by Alcohol Monitoring Systems, Inc. The device, worn as an ankle bracelet, is commercially available to law enforcement agencies and privately operated correctional institutions. It is primarily designed and marketed for court-ordered alcohol monitoring of Transdermal Alcohol Concentration (TAC) readings.<sup>11</sup>

SCRAMs have limitations. TAC does not directly correlate to blood alcohol concentration in a SCRAM.<sup>12</sup> SCRAMs are useful in general population biomonitoring of self-induced alcohol consumption as a passive preliminary testing device. The manufacturer's criteria conveys SCRAM can reliably detect the consumption of five or more standard beers or drinks, and 45.9% of all occasions of drinking one to three beers went undetected when using SCRAM's 0.02g/dl as a threshold.<sup>13-15</sup>

SCRAM offers litigation support to consumers of its products through expert witness services. SCRAM's "judicial liaison" testified as an expert witness concerning the credibility and reliability of SCRAM CAM SMO2 results in *U.S. v. Colby*.<sup>16,17</sup>

Based on the *Colby* record, SCRAM's "judicial services liaison" possess controvertible expert witness qualifications consisting of: being an attorney; in-house administrative experience as a business product manager; conducting product service and promotional lectures; and completing two SCRAM operator training courses. SCRAM's expert severely lacks expected credentials: a formal science education; specialized knowledge; demonstrable expertise; any publications; scientific memberships; and published subject research.

Selected random *Colby* issues include: (1) limited knowledge of the fuel cell; (2) calibration standards and procedure not subjected to scientific scrutinization and criticism; (3) questionable basis of published analytical tolerance; (4) yearly calibration standards not maintained; (5) asserting average fuel cell life span is less than a year conflicts with SCRAMS annual calibration; (6) device diagnostics being irrelevant to calibration; (7) fuel cell and pump degradation only issues adversely affecting accuracy; (8) exhaust/contamination test conducted without supporting data; (9) misdirected authentic peer-review studies and topics (transdermal studies not SCRAM studies); (10) false positive authenticity rate less than 1%; (11) SCRAM studies conducted on earlier generation devices or not using SCRAM analytical software or analysts to determine positives and negatives; and (12) SCRAM lowered testing standards for confirmed positives several times over the years. Missing were relevant peer-review studies discussing revised methodology requirements, false positive rates, current cited cases, controlled testing for false positives, and actual situations with double-blind testing.

Initial concern is whether the SCRAM device is accurate and reliable to identify and measure TAC.<sup>18,19</sup> SCRAM seemingly presents tenuous and dubious expert witnesses and selective marketing data without adhering to governmental or industry reliability standards. The perceived lack of quality, candor, and competent expert witness testimony reasonably corroborates SCRAM's diminished product stature, controversial evidential value, and invites professional sanctions for its expert witness. Competent oversight and greater scrutiny should be used.

Limitations of SCRAM devices and TAC data is arguably skewed or specious through marketing and other claims, including shaped and shaded judicial liaison testimony. Apparently, public policy against alcohol intoxication cases continues to detrimentally transcend expert witness ethics, qualifications, and competency.

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4. Gil Sapir. Qualifying the Expert Witness: A Practical Voir Dire. *Forensic Magazine*, vol.4, no.1, February/March 2007, p.30.
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6. *Murphy v. AA Mathews*, 841 S.W.2d 671, 674 (Mo. 1992).
7. *Marrogi v. Howard*, 805 So.2d 1118, 1124-1125, 1128 (La. 2002).
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### SCRAM, Expert Witness, *Daubert*



### **F22 Effects of Ignition Interlock Laws on Driving Under the Influence (DUI) Fatalities**

*Michael Miller, MFS\*, National University, La Jolla, CA 92037; Ismail M. Sebetan, MD, PhD\*, National University, La Jolla, CA 92037-1011; Vivian L. Stafford, MFS, San Diego, CA 92138; Paul Stein, PhD\*, National University, La Jolla, CA 92037*

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**WITHDRAWN**



### F23 The Significance of Informed Consent in Workers' Compensation Forensic Blood Toxicology Testing

Helen D. O'Connor, LLM\*, Robert O'Connor, Jr., Houston, TX 77056-8187

**Learning Overview:** This presentation will inform attendees of the significance of informed consent in drug/alcohol forensic testing with relation to workers' compensation statutes, state and federal constitutional privacy protections, the United States Health Insurance Portability and Accountability Act (HIPAA) statute, and also the medical ethical principle of *primum non nocere* in the preparation and disclosure of a workers' compensation forensic science expert report.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the legal and ethical support for the requisite of informed consent of blood samples used in workplace injury forensic science analysis.

Informed consent is a process by which a patient (who is legally of the age of majority) and medical care providers document the agreement under which medical care, treatment, or services are provided to the patient. If an adult patient is competent, then consent for treatment must be obtained by the medical care provider and documented in writing. In emergency situations, when the patient does not have the legal competency to properly give consent for medical treatment, the consent is implied to protect the emergency medical provider under the "Good Samaritan" laws. In the case of minors, the authorized legal representatives (usually the custodial parent/s), are authorized to give consent for the medical care and treatment of the minor patient.

Under workers' compensation statutes, there is a defense/bar for the insurer of the subscriber to provide benefits to the injured worker if the circumstances of the workplace injury occurred when the worker was "intoxicated," whether under the influence of alcohol or other drugs. The legal definition of "intoxication" is set forth under the relevant workers' compensation statute and is proven by forensic science testing of either urine or blood samples from the worker at the time of the injury incident. The focus of this presentation is on blood sampling forensic science expert reports, with analogous principles applicable in urine testing. The effect of forensic science testing of worker blood samples can foreclose the possibility of treatment and monetary benefits to the worker for his workplace injury if the incident occurred while the worker was in a state of "intoxication." An analogy is the criminal sanction for drivers who operate vehicles while in a state of "intoxication," which can include confinement, loss of driving privileges, and/or monetary penalties.

The worker/patient's blood testing is part of the Protected Health Information (PHI) under the United States HIPAA statute. The United States HIPAA statute requires health care providers to protect PHI from unauthorized disclosure, including drug testing in any patient/injured worker, and mandates documentation of consent by the worker/patient for any disclosure.

The PHI information of the worker/patient involves state and federal constitutional guarantees of "privacy," which require that the worker/patient must have executed a proper consent for the "intoxication" testing to be performed by the medical care provider, including: the sampling, the laboratory processing of the specimen, the toxicology analysis, and the disclosure of such forensic science results in an expert report.

The consent requirements ensure statutory and constitutional protections for workers/patients for their medical care and treatment—including forensic science analysis and reporting of blood sampling to workers' compensation subscribers and their insurers. Informed consent also encompasses the medical ethical principle of *primum non nocere* because the worker/patient will be harmed by deprivation of treatment and compensation if informed consent is absent for the forensic science analysis disclosure in the workers' compensation case. Hospital toxicology lab testing routinely notes the limitation of its use "for medical purposes only."

The principles of informed consent in the workers' compensation worker/patient scenario are discussed with specific relevant Texas statutes and constitution, and United States Constitution privacy protection in reported case law.

In the United States, proper consent must be in place for disclosure of the worker/patient blood alcohol/drug forensic science expert analysis in court/administrative hearings.

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#### Consent, Jurisprudence, Ethics



### F24 Genetic Genealogy and Law Enforcement: New Bedfellows

*Donald E. Shelton, JD, PhD\*, University of Michigan-Dearborn, Dearborn, MI 48128-2406*

**Learning Overview:** After attending this session, attendees will have a better understanding of how genetics and genealogy have come together to solve crimes and will also learn how recent developments raise both legality and ethical issues.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by making practitioners aware of how genetics and genealogy can now be used by law enforcement, as well as the legal and ethical issues that are presented by the process.

Since the identification and arrest of the suspect in the “Golden State Killer” investigation in early 2018 using novel DNA and genealogy tools, dozens of law enforcement investigators in North America have effectively used such methods in high-profile cold cases. A combination of advanced DNA methods, using thousands of Single Nucleotide Polymorphisms (SNPs) along with searching of ancestry databases, has allowed genealogists to assist investigators in identifying possible persons of interest as suspects in unsolved crimes. A free website database called GEDMatch allows comparison of suspect DNA to any of the millions of persons who have voluntarily submitted their DNA to that website database to accurately identify distant relatives of the suspect. In the past year, some of the more general direct-to-consumer genealogy websites, such as FamilyTree® and 23andMe®, have also indicated that they will release information to law enforcement under certain conditions. These tools have significantly changed the face of genealogical searching, and those changes now offer new investigative methods of crime solving.

Police can submit raw data from crime scene DNA to one of the new genealogy databases to find relatives of the person who left the specimen. While the suspect may not be in the Combined DNA Index System (CODIS), and may not have submitted his or her DNA to the genealogy site, the DNA of any of his or her relatives who have innocently submitted their data for genealogy search purposes can lead police to a range of suspects. The results of DNA-based genealogy searching allows follow-up investigation by police to further narrow the range of suspects based on age, locale, and other factors associated with the crime. Once a possible suspect has been identified, police can obtain a known DNA sample from the suspect. This can be done using discarded DNA without a warrant (for example, a discarded tissue from a garbage can in the “Golden State Killer” case). After that DNA profile is related to the crime scene specimen, the police have the basis for a warrant.

The entire process may raise potential admissibility issues that have yet to be decided by the courts. These admissibility issues involve questions such as legal standing and waiver doctrines, as well as the more basic issue of whether the ancestral leads generated by the process are even relevant to introduction of the eventual DNA comparison of the crime scene DNA to the defendant’s DNA. The waiver issue has recently become more prominent since GEDMatch and some of the more general ancestry searching sites have now required an “opt-in” consent before data will be shared with law enforcement.

Notwithstanding the legality issues, the use of genealogical data raises ethical and policy issues. There is a significant debate about whether individuals who submit their DNA for personal genealogy research understand that it may expose their relatives to potential criminal prosecution. The recent “opt-in” requirements are attempts to insure that true “informed consent” is obtained before the information is released to law enforcement. Furthermore, some argue that the very concept of genealogical searching by law enforcement is the beginning of a “slippery slope” leading to a “police state” mass identification of the populace that violates basic societal notions of privacy. Others, however, maintain that consensual submission of DNA data that may lead to the apprehension of a dangerous criminal is an entirely ethical and individual decision.

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#### Genetic Genealogy, Genetic Genealogy Admissibility, Genetic Genealogy Ethics



### F25 You Are the Judges: An Interactive Session on Cutting Edge Issues at the Intersection of Law and Science

W. Milton Nuzum III, JD\*, Supreme Court of Ohio, Columbus, OH 43215; Stephanie Domitrovich, JD, PhD\*, Sixth Judicial District of PA, Erie, PA 16501

**Learning Overview:** After attending this presentation, attendees will better understand the role of judges as gatekeepers when cutting edge science arrives at the court's doorsteps and be able to apply rules of evidence to scientific issues in actual court cases involving expert witnesses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with learning opportunities to be able to apply and understand rules of evidence involving their roles as expert witnesses in the courts through the use of actual court cases.

This presentation will first provide a quick primer on the evolution of admissible scientific expert testimony. This presentation will review how judges make critical admissibility decisions as gatekeepers of scientific evidence in their courtrooms. State jurisdictions can range from *Frye* to *Daubert* jurisdictions. *Frye v. U.S.* is a one-page decision that still exists in several state jurisdictions.<sup>1</sup> Under the *Frye* test, expert opinions based on scientific techniques are admissible only when the techniques are generally accepted as reliable in the relevant scientific community. *Daubert v. Merrell Dow Pharmaceuticals, Inc.* is the leading case regarding this gatekeeper role of admissibility decision making.<sup>2</sup> The United States Supreme Court in *Daubert* defined the judge's role as a gatekeeper to admit relevant and reliable scientific knowledge by reviewing a non-exclusive list of questions for "Gatekeepers": Has theory or technique been tested? Has it been subjected to peer review and publication? Is there known or potential rate of error in technique? Is it generally accepted in the relevant scientific community (the *Frye* test)? Federal Rule of Evidence 702 permits witnesses qualified by knowledge, skill, experience, training, or education to testify if: (1) the expert's scientific, technical, or other specialized knowledge will help the trier of fact to understand the evidence or to determine a fact in issue; (2) the testimony is based on sufficient facts or data; (3) the testimony is the product of reliable principles and methods; and (4) the expert has reliably applied the principles and methods to the facts of the case.

This presentation will also review actual cases with attendees through an interactive PowerPoint® vehicle for attendees to respond to on admissibility issues. This presentation has selected various state and federal cases to review the admissibility considerations as to various cutting edge issues. How do courts determine the admissibility of cutting edge issues, such as cases involving the STRmix™ program? What are the considerations in discovery? Is the program in the exclusive control of the prosecution team? Can the prosecutor be compelled to produce the STRmix™ software program, the program source code, and internal validation studies? Can computer algorithms be compelled to testify? This presentation will review recent cases involving pathology, toxicology, and chemistry. This presentation will review cases involving qualifying experts. What are the considerations that courts find important in qualifying experts? What about the methodology of the expert? Does Forensic Statistical Tool (FST) testing of DNA meet the *Daubert* standard of admissible evidence? In the case of hypergeometric testing, when is the scientific mythology sufficiently reliable to admit? What about authenticating Facebook® postings where the case relies solely on Facebook® postings? Are these postings admissible when an expert has been qualified? What proof is necessary to authenticate social media evidence, such as Facebook® postings and communications? Additional cases will be reviewed as time permits.

**Reference(s):**

<sup>1</sup> *Frye v. United States.*, 293 F. 1013 (D.C. Cir. 1923)

<sup>2</sup> *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579 (1993)

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**Courts, Evidence Rules, Cutting Edge Issues**

## F26 The Importance of Evidence in Proof of Accusation in Criminal Procedure Law in Turkey

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**Learning Overview:** The goal of this presentation is to propose a solution to the problems in the judicial process that are encountered in obtaining evidence for Proof of Accusation in Criminal Procedure Law in Turkey.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how the crime is not rendered suspect when the issue of proof comes into play. The function of the Law of Criminal Procedure, criminal and criminal norms by showing the offender in return of actual execution of the application if it has been exercised to determine by whom it is handled. Essentially, the goal is to investigate material facts. In order to achieve this goal, it must be proven that a crime was committed by acts as shown.

In the legal sense, a crime, criminal, or legal enforcement of security is measured by the institutions of a society. The person who performs the crime is called a criminal. The history of crime is as old as the history of humanity. Throughout history, governments and society are required by the dynamics of the committed criminal action that perpetrators be judged and it is concluded through the trial of the perpetrator that, if guilty, is subject to sanctions in various ways. If it is concluded he did not commit the crime, he is acquitted.

That the crime is not rendered suspect by the issue of proof comes into play. Thus, the suspect is innocent or prove to himself that will be hard to prove that the party who claims to have committed a crime. In Roman law, put some money in our legal system as it is the party that has to prove the claim. In medieval Europe, he had to prove that the suspect is innocent. The function of the Law of Criminal Procedure, criminal and criminal norms by showing the offender in return of actual execution of the application if it has been exercised to determine by whom it is handled. Essentially the goal is to investigate material facts. In order to achieve this goal, it must be proved that a crime was committed by who acts as shown. For punishment to follow authority who has the authority, by whom the crime does not have the authority to decide that arbitrarily, the proof of this must be done in accordance with the law. Truth is relative. Naturally, we go ways to influence the court, the parties, the judicial authority, he would go the way of finding the truth by examining the evidence. In criminal proceedings, the Turkish Criminal Procedure article 217/2 of the law to regulate the discretion of the judge as the “alleged crime, it can be proven with any evidence obtained in accordance with the law” in the form of are arranged. The judgment of events that have occurred during the criminal procedure shall be proved before the time of the event and the conditions of the emergence of prior knowledge for reasons such as lack of freedom of evidence in criminal procedure was adopted that principle asserts. In this presentation, the problem of evaluation of evidence that is required for proof of the allegations will be discussed.

Therefore, search for truth cannot be executed at all costs. This process should take into consideration the rights that human beings intrinsically have. The thesis therefore consist of the parts as follows: purpose of the criminal process and historical evolution of it; definition, gathering, and reviewing process of evidences.

### **Criminal Process, Juveniles, Evidence Law**





## F27 The Need for a Full Specification for Digital Forensic Tool Validation

Nicolas R. Hughes, JD\*, Harris County Public Defender's Office, Houston, TX 77002-1957

**Learning Overview:** After attending this presentation, attendees will better understand the definition and process of validating digital forensic tools.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the need for a fully specified definition of the validation of digital forensic tools.

Digital forensics encompasses the acquisition and analysis of digital data. Digital forensic examiners collect and analyze evidence pertinent to the investigation of traditional crime, cybercrime, or civil matters. Examiners routinely rely on software- and hardware-based tools to analyze otherwise unmanageable amounts of data.

Tool validation is the recognized process for evaluating the accuracy and reliability of an instrument. Validation requires the "provision of objective evidence that a given item fulfills specified requirements, where the specified requirements are adequate for an intended use."<sup>1</sup> The discipline of digital forensics provides a definition of validation that does not fully capture the intent of the formal definition: "[a]n evaluation to determine if a tool, technique or procedure functions correctly and as intended."<sup>2</sup>

In digital forensics, confusion about what "validation" means results in the use of the term to describe a wide range of methodologies. The term may describe manufacturer's internal studies, manual verification of data, and fitness-for-purpose testing conducted by the Computer Forensics Tool Testing Program or laboratories.<sup>3</sup> Non-public or limited manufacturer studies do not provide the empirical data needed to justify reliance upon the tool. Fitness-for-purpose testing and manual verification may demonstrate that a forensic tool operated successfully in one scenario, but typically fails to demonstrate performance over a full range of realistic conditions.

Validating digital forensic tools is inherently difficult. One difficulty is accounting for the number of variables affecting digital investigations. Many tools perform multiple functions—for example, correlating text messages, Global Positioning System (GPS) data, and photo metadata. Tools may accommodate different operating systems, devices, and hardware configurations. Tools and software often receive updates. Each different function, hardware configuration, and version represents a variable that may affect a tool's operation. Additionally, collecting digital forensic reference samples is difficult. The sensitive personal information present on digital devices poses privacy and legal concerns, making it challenging to use real samples.

The discipline should provide a functional, low-level specification of tool validation defining the requirements, reference samples, and methodologies used during tool validation. First, the discipline should enumerate the specifications and requirements for tool validation. These should include the range of conditions expected during analysis, a detailed hierarchy of functionality possible for each forensic process, and any known anomalies expected to affect a given forensic process.<sup>1,4,5</sup> Second, the discipline should curate appropriate validation methods and reference samples. The discipline should specify the appropriate use of a validation method, the provenance of reference samples, how to generate synthetic evidence, and should specify how to structure data generated during the study. Finally, the discipline should identify remaining gaps in the assurance methodology. The gaps mark the limitations of present knowledge about forensic tools and should direct future efforts.

Fully specifying the definition of digital forensic tool validation is beneficial. A fully specified definition permits researchers, experts, and developers to make independent, incremental contributions to a common body of assurance methodology. It establishes a common definition of tool validation. Finally, a full specification encourages the generation of public, transparent data used to justify conclusions about tool performance. By fully specifying the definition of tool validation, the discipline strengthens the foundation of digital evidence.

### Reference(s):

1. BIPM. *International Vocabulary of Metrology—Basic and General Concepts and Associated Terms (VIM)* 3rd edition. 2008.
2. SWGDE. *SWGDE Recommended Guidelines for Validation Testing*. 2014.
3. NIST. *Computer Forensics Tool Testing Program (CFTT)—Software Quality Group*.
4. Guo Y., Slay J., and Beckett J. Validation and verification of computer forensic software tools-searching function. *Digital Investigation* 6 (2009): S12-S22.
5. Lyle, James R. If error rate is such a simple concept, why don't I have one for my forensic tool yet?" *Digital Investigation* 7 (2010): S135-S139.

### Digital Forensics, Tool Validation, Specification



## F28 #Datastories

*Paul Reedy\*, 4th Street Global, Washington, DC 20003*

**Learning Overview:** After attending this presentation, attendees will understand the increased challenges of digital evidence and hear an alternative approach to meet the challenge.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an alternative approach to meet the increasing challenge of digital evidence as new technologies and applications become available in the consumer and business marketplaces.

As the volume of forensic data continues to grow, alternative approaches to assessing and interpreting the data are required. One alternative is presented that attendees may wish to adopt.

A different approach to looking at data is required. All forensic information is data, no matter from where it is derived. The most obvious is digital evidence obtained from data storage devices; however, in many investigations, this now includes network and internet logs. But what are we to make of DNA, or images of latent fingerprints, or those related to firearms? How does one consider financial transaction records? Gone are the days of the digital forensics and other experts looking at the data in isolation. A different approach is the use of multidisciplinary teams looking at the whole of digital evidence, but also interpreting other forensic data when available. Teams comprising expert individuals who bring a range of skills and knowledge to bear on the question(s) at hand, who can read and interpret different forms of data from multiple sources. In collaboration, the data story can be revealed.

Every person has a unique digital identity and, as technology evolves, this identity will become more specific and more informative. Historically, the challenge has been about getting hold of the data. Where is it located? Is it encrypted? Is it hidden? Can we look at all of it?

It is no longer just the phone and computer. Now, as the Internet of Things becomes ubiquitous, every individual is part of the digital mesh, permanently integrated into the system 24 hours per day, 7 days per week. An individual's every thought, behavior, relationship, decision, and action is captured digitally. Now, there is too much to look at all of it, and it is all in different formats, from different sources, different devices, and generated for different purposes.

4G telecommunications brought innovations that were unanticipated. Initial marketing was for more reliable phone calls and faster data transfers. What was not expected was the explosion in social media; Netflix®, Hulu®, and other streaming services; the beginning of autonomous vehicles. Although not directly related to 4G, technological evolution has resulted in the appearance of deep fakes and the weaponization of social media has real-world impacts. Is the data real?

As 5G telecommunications with increased speed (download a high-definition movie in less than five seconds), improved latency to almost assure autonomous vehicle safety, and improved reliability, what will be the challenges for those of us who are looking at digital evidence to ascertain the course of events and the person involved in an incident. New applications that can be anticipated will include telemedicine with implanted devices and monitors, building access and control management, public transport management, and motor vehicle monitoring and management. The total volume of data will continue to grow incrementally as 5G is rolled out, the infrastructure is upgraded and new, yet to be conceived, applications are developed and marketed. To illustrate the point, BEBO was the largest social networking site in the United Kingdom with 10.7 million users and sold to AOL® for \$850 million in 2008. In 2010, AOL® decided to either sell it or close it down as it was losing users to Facebook®. That was just ten years ago. This was around the same time as the advent of 4G.

**#datastories, #digitalevidence, #forensicdata**



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## F29 Spotting Stingrays: The Legal Issues of Covert Cell Phone Location Surveillance

*Michael Buresh, JD\*, Cook County Public Defender, Chicago, IL 60602*

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**Learning Overview:** The goal of this presentation is to introduce attendees to cell-site simulators, a covert cell phone location tracking device commonly referred to as a “stingray.” The presentation aims to teach attorneys how to identify when a stingray has been used against their clients and how to seek legal recourse. This presentation will also cover the current state of search-and-seizure law on stingrays. Two real-life case examples in which law enforcement utilized stingrays will be presented to provide specific examples and to contextualize the presentation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining how attorneys can identify cases in which a stingray may have been used and how to uncover evidence of stingrays.

Cell phones communicate via stationary cellular antennae (cell sites, also commonly referred to as “cell towers”) that relay phones’ radio signals over long distances. Law enforcement can exploit this technology to conduct covert surveillance of citizens and their locations via a device called a cell-site simulator (a.k.a. “stingray”). Like “Kleenex<sup>®</sup>” or “iPad<sup>®</sup>,” the term “stingray” derives from a specific brand of cell-site simulator manufactured by Harris Corporation. A stingray impersonates a real, carrier-operated cell site and forces all phones in the immediate vicinity to connect to it. The stingray then acts as a go-between, relaying signals between those target phones and the true, carrier-operated cell sites. These stingrays can decode these signals to reveal the identifying information of the phone (the International Mobile Equipment Identifier [IMEI]) and the identifying information of the customer (the International Mobile Subscriber Identifier [IMSI]). A stingray can act as a pen register/trap and trace device by intercepting all incoming and outgoing phone numbers to and from the target phone. A stingray also acts as a wiretap by intercepting the content of voice calls and text messages to and from the target phone. However, the most commonly used ability of a stingray is its ability to track the location of the target phone by measuring signal direction and strength. Stingrays can track multiple phones simultaneously and identify a specific individual’s phone by the process of elimination.

As a covert surveillance device, law enforcement’s use of these devices has been shrouded in mystery. Law enforcement agencies obtain these devices under strict Non-Disclosure Agreements (NDAs) with the Federal Bureau of Investigation (FBI). These NDAs prohibit local police from disclosing the existence or use of their stingray without the express permission of the FBI. The NDAs even commit local law enforcement to dismiss cases at the direction of the FBI if backed into a corner by a court. Police use vague language, parallel construction, legal fictions, and even outright lies to conceal their use of stingrays in criminal investigations.

The presentation will cover the current state of the law on stingrays. While there is no directly applicable United States Supreme Court opinion on the specific topic of stingrays, several lower courts and state legislatures have weighed in on the issue. This presentation will also cover two specific case examples in which clients of the presenter were “stung” and in which law enforcement went to great lengths to conceal their warrantless use of stingrays. In these cases, it was ultimately the work of independent government accountability and transparency activists (and their attorneys) that uncovered Chicago law enforcement’s ownership and use of stingrays and secured justice for the clients.

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### Stingray, Cell Phone, Surveillance



## F30 Historical Cell-Site Location Basics

Michael Buresh, JD\*, Cook County Public Defender, Chicago, IL 60602

**Learning Overview:** The goal of this presentation is to teach attendees the basic principles of historical cell-site location information and the current state of the law on cell-site location. This presentation will introduce attendees to various types of cell-site location data, the various records that comprise cell-site location data, and the legal issues implicated by the introduction of cell-site location into evidence in court.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by spreading knowledge of, and building competence in, a relatively unknown field of forensic science. The case example will serve as a cautionary tale for both cell phone location surveillance practitioners and attorneys as to the potential consequences of not fully understanding this discipline.

Cell phones rely on stationary cellular antennae (cell sites, also commonly referred to as “cell towers”) that relay phones’ radio signals over long distances. Cellular service providers keep records of which specific cell sites transmit their subscriber’s calls and text messages for the purposes of both billing and network analysis. Law enforcement can obtain these records via warrant to determine individuals’ past locations for use in investigation or prosecution. These records include Call Detail Records (CDRs), tower lists, and CDR keys, and are commonly referred to as Historical Cell-Site Location Information (HCSLI). Inadequately trained law enforcement officers can make errors in their analysis and often overstate the precision and, therefore, the probative value, of this evidence. It is important that practitioners and attorneys are aware of these potential pitfalls in order to avoid miscarriages of justice.

The legal landscape of HCSLI is evolving rapidly. Prior to June 2018, law enforcement commonly obtained HCSLI via a “d-order” under the federal Stored Communications Act (SCA). This statute allowed law enforcement to obtain these records with a court order issued on an evidentiary standard lower than probable cause. In a landmark ruling, *Carpenter v. United States*, the United States Supreme Court declared that individuals possess a reasonable expectation of privacy in their past locations as revealed through HCSLI.<sup>1</sup> The Supreme Court also held the SCA procedure for obtaining HCSLI to be invalid and ruled that law enforcement must generally get a warrant to obtain these records. While the privacy interest in these records is now established, the lower federal courts and state courts are still developing the law around other aspects of cell-site location, such as the exclusionary rule, real-time cell-site location, and tower dumps.

In 2016, while investigating a near-fatal shooting, Evanston, IL, police officers obtained the HCSLI of a suspect, C.L., by his consent. In analyzing C.L.’s HCSLI, a detective made a crucial error that resulted in C.L. being arrested and charged with attempted murder. C.L.’s defense attorneys were able to identify this error and referred the case to an independent expert who was able to refute the erroneous analysis. This presentation will explain the error made in C.L.’s case, discuss other common errors in cell-site location analysis, and prepare attorneys to identify and counteract these errors.

### Reference(s):

<sup>1</sup>. *Carpenter v. United States*, No. 16-402, 585 U.S. \_\_\_\_ (2018).

### Cell Phone, Cell Site, Surveillance



### F31 Drone Laws: The Imminent Quandry

Corey A. Bauer, JD\*, *Bauer Law, Pittsburgh, PA 15222*; Victor W. Weedn, MD, JD\*, *The George Washington University, Washington, DC 20007*; Anthony M. Hallett\*, *Unmanned Response, Inc, Beaver, PA 15009*

**Learning Overview:** After attending this presentation, attendees will understand: (1) the early stages of development of the civil and criminal law of Unmanned Aerial Vehicles (UAVs); (2) the conflicts between federal and local laws; and (3) how privacy implications are substantial.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an awareness of the issues that are developing from the proliferation of drones.

The civil and criminal law of UAVs is in an early stage of development. Federal and local laws often conflict. Privacy implications are substantial. The Federal Administration (FAA) has adopted the term Unmanned Aircraft (UA) for aircraft systems without a flight crew on board, but they are more popularly known as UAS, or simply drones. UAS are approaching ubiquity in America's skies and, as is typical of technological innovation and diffusion, the law lags behind. The FAA currently permits drones under 55 pounds to be operated for two distinct purposes—recreational or commercial—each of which must follow a different set of rules that periodically change. Also, it is against federal law for anyone, including local law enforcement, to shoot down or disable any manned or unmanned aircraft. Can the FAA enforce their UAS laws, or do they have to rely on local law enforcement? The FAA has been attempting to assert itself as the sole regulator of the airspace—*field preemption*, but state laws and local ordinances proliferate in the face of unenforced federal preemption. States press their historic police powers, reserved to them by the United States Constitution, as well as primary land use, zoning, privacy, and trespass jurisdiction of launch/landing sites. Most of the federal regulations involve air safety, and most local laws involve privacy, but the proposed and enacted local laws vary widely. An example state law is 18 Pa.C.S. § 3505, which criminalizes the use of drones to: (1) conduct surveillance upon people in a private place; (2) operate so as to place another in reasonable fear of bodily harm; or (c) deliver, provide, transmit, or furnish contraband to convicts in prison or inmates in a mental hospital. Outside of breaking the aviation rules themselves, drones are primarily used in ways that may invade our privacy.

Beyond the small toy drones, most UAS are equipped with cameras. The United States Supreme Court has already ruled in *California v. Ciraolo* and *Florida v. Riley* that there is no reasonable expectation of privacy from the air.<sup>1,2</sup> Even if aerial observation was considered a privacy infringement, would merely racing over the property with a camera at 20mph be a violation? A recent criminal case from northwest Pennsylvania (among the first in the country) illustrates the current problematic state of affairs. This case involves the attempted prosecution of a recreational drone operator for allegedly disturbing dogs at a K-9 training kennel. The drone log was used by the defense to challenge the assertions of the local authorities. In 2017, the FAA launched the UAS Integration Pilot Program (IPP), a collaboration of local, state, and federal government agencies and private-sector vendors and operators. The intent is to promote cooperative oversight of the national airspace. Progress has been encouraging, but is universal agreement and adoption even possible? Furthermore, consider the American National Standards Institute's Roadmap for UAS, an ongoing collaboration that has identified 60 "gaps" in UAS oversight that must be remedied through new regulations issued by dozens of federal agencies. Even the FAA will soon add another layer of complexity when drones are widely approved to fly beyond the operator's visual line of sight. Ambiguities, preemption conflict, and the inherent intrusive nature of drones will almost certainly provoke a flood of unwarranted cases in the months and years ahead.

**Reference(s):**

- <sup>1.</sup> *California v. Ciraolo*, 476 U.S. 207 (1986).
- <sup>2.</sup> *Florida v. Riley*, 488 U.S. 445 (1989).

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#### UAVs, Drones, Privacy Law

## G1 The Applicability of the “Dimodent” Sex Predictive Equation Assessed in a Senegalese Population

Khalifa Dieng, DDS, PhD\*, Dakar, SENEGAL; Sankoung Soumboundou\*, University Cheikh Anta Diop, Dakar, 99000, SENEGAL

**Learning Overview:** After attending this presentation, attendees will gain a method to achieve a sexual dimorphism using odontometric data specific to each population. The purpose of this study is establish the degree of this dental dimorphism within the population.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the determination of gender is one of the most important and crucial steps in identification using the Dimodent method described by Fronty.<sup>1</sup>

**Materials and Methods:** A total of 104 subjects (52 males and 52 females) aged 20 to 60 years were included in this study. Mesiodistal and vestibulo diameters, lingual of the mandibular lateral incisor and canine, were measured with a digital caliper on plaster models. The measurements obtained were integrated into the sexual prediction equation:  $P=1/(1 e^y)+Y$ . Y is evaluated based on measurements of mesiodistal and buccolingual diameters. The Mesiodistal Dimension (MD) was defined as the largest distance between the contact points on the proximal surfaces of the dental crown.

$P=1/(1 e^y)+Y$  is evaluated according to measurements of mesiodistal and vestibulo diameters.  $Y=+24.2 (1.54*+I MD) (1.92*-I VL) (2.84* C-MD) (3.38*C VL)$  (I=Incisor, VL=Vestibulo-Lingual, C=Canine). The equations and P and Y were created on Excel<sup>®</sup> thanks to the inter- and intra-observer variabilities that were evaluated with the Kappa Cohen test.

After casting alginate (irreversible hydro-colloid) impressions with hard plaster, the measurements were taken along the long axis of each tooth, with a digital caliper with a digital display of precision of 0.01mm according to the method described by Dimodent.

This study included 104 Senegalese melanoderm subjects, including 52 women and 52 men randomly selected, with an average age of 41.22 years  $\pm$  12.58. The study of inter- and intra-writing of the functions  $P=1/(1 e^y)+$  where P expresses the probability of being in the presence of a feminine or masculine dentition. According to Dimodent: if P is between 50% and 100%, the teeth are probably female. If P is between 0% and 49%, the teeth are likely to be male. Depending on the value of P found, the samples were ranked according to the pattern.

The collected data were analyzed using Microsoft<sup>®</sup> Excel<sup>®</sup> (version 2013) software. The comparison of the data was made with the Chi-square Xtests2 and Pearson and a significance level  $p \leq 0.05$  was retained.

**Results:** The study of inter- and intra-examiner variability yielded a satisfactory kappa of 0.70. A statistically significant difference was found in the medial-distal ( $p<0.0001$ ) and vestibul-lingual ( $p<0.0001$ ) diameter of the canine. The measurements obtained on the lateral incisor, mesiodistal diameter, are not discriminate.

In this study, the overall positive prediction rate is 86.53%. The success rate was higher for men (90.38%) than for women (82.69%).

**Conclusion:** The method used in this study is simple and inexpensive to conduct and therefore can be applied in forensic odontology to establish an individual's sexual identity.

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### Sex Assesment, Dimodent Equation, Mandibular Canine



## G2 A Case of Great East Japan Earthquake Human Remains for Which Digital Imaging Analysis of Dentures Helped in Identification

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**Learning Overview:** After attending this presentation, attendees will have acquired a new dental identification technique.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that judgment of a case report of comparison was based on very little evidence utilizing artificial teeth from dentures.

Human remains were discovered immediately after the Great East Japan Earthquake and were cremated, although the person was not identified. The full set of dentures in the mouth at the time of discovery of the remains were kept as possessions of the victim in the municipality. Possible identification of the remains emerged through collation with a DNA database, but an additional personal identification method was desired for a more reliable identification. Eight years later, the dental clinic that the candidate attended during his lifetime was identified, and it was revealed that the dental clinic prepared the upper and lower full dentures. Moreover, it was revealed that the dentures the candidate wore had rare artificial teeth in the molar regions.

Demonstration of wearing the dentures prepared with the rare artificial teeth by the unidentified remains may be a big help in judging the comparison. Thus, this study tried to increase the possibility that the remains were the patient (candidate) who attended the dental clinic by analyzing whether the artificial teeth of the dentures worn by the remains were the rare artificial teeth.

Images of the dentures belonging to the unidentified body were acquired using cone beam Computed Tomography (CT) and Stereolithography (STL). Then, the artificial teeth used in the dentures worn by the candidate, Livedent FB20 Plastic 108, and hard resin teeth used about 40% of the time in Japan (frequently used in general dentures prepared within the coverage by national health insurance and used in the anterior tooth region of the dentures prepared by the dental clinic), and ENDURA S28 A3, used as a control material, were imaged using a desktop scanner for dental technicians and STL was constructed. These were superimposed on STL of the bilateral upper and lower first molars of the artificial teeth in each of aforementioned images. The software used for superimposing the images was spGauge. As a result, it was determined that the artificial teeth of the dentures worn by the unidentified body were very likely to be identical to those used by the candidate.

Component analysis was also a measure for this case, but items left behind by an as-yet unidentified individual cannot be destroyed, so non-destructive analysis was required. In the analytical results, large differences were noted in the functional palatal- and lingual-side cusp between the artificial teeth of the dentures worn by the remains (candidate) because the morphology of these is likely to have changed due to being ground for occlusal adjustment by a dentist when new dentures are attached and attrition by mastication, but almost no difference was noted on the non-functional buccal surface or fissure of the occlusal surface of the cusp; these useful results helped in obtaining a judgment of the comparison.

### Disaster Victim Identification, Forensic Odontology, Stereolithography



### G3 Should Facial Marks Created Because of Tooth Loss Be Considered as Hard Evidence?

*Huseyin Afsin, PhD\*, Istanbul 3400, TURKEY; Gulnaz T. Javan, PhD, Alabama State University, Montgomery, AL 36104*

**Learning Overview:** After attending this presentation, attendees will be familiar with different views on using facial marks due to tooth loss.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing another way to use facial marks due to tooth loss in forensic cases.

Opinions on using facial marks resulting from tooth loss as hard evidence vary. This study attempts to evaluate different opinions on using facial marks due to tooth loss as evidence according to Turkish penal code.

This study brings together opinions and applications of facial marks due to tooth loss by forensic experts, dentists, and jury members as a piece of evidence used in a usual course of their practice. Data has been analyzed and evaluated by a statistical program.

A total of 162 people participated in this study, which included 103 forensic experts/assistants, 38 jurists, and 21 dentists. Of these, 38.8% of the forensic experts/assistants, 90.5% of the dentists, and 34% of the jurist participants in the study were professionals practicing in three big cities of Istanbul, Ankara, and Izmir. Just over 60% (61.1%) of participants had less than 10 years of experience, while 38.5% had more than 11 years of experience in their respective professions. Even though the number of participants with less than 10 years of experience in the study is statistically higher, the majority of the dental participants had more than 11 years of experience ( $p < 0.05$ ). Thirteen percent of the study subjects were categorized as having no face marks because of tooth loss and 87% had definite facial marks as a result of tooth loss. In categorizing study subjects, there was no statistical significance among participants according to their areas of expertise or years of experience ( $p < 0.05$ ). Participants were grouped according to their area of expertise and written opinions.

Tooth loss can be repaired close to its original aesthetic appearance with today's dental technologies that are available to dentists. Despite this advance in dentistry, people with tooth loss have to live the rest of their lives with dentures or without replacing the tooth/teeth at all. There are several reasons that contribute to one's decision to not seek dentist's help; major contributors include fear of dental treatment, economic reasons, lack of access to dental services, and transportation problems. The importance of evaluation of facial marks due to tooth loss and its application are what this study hopes to highlight.

#### **Tooth Loss, Facial Marks, Evidence**





### G4 Paradise Lost: The Camp Fire

*Mark D. Porco, DDS\*, Lincoln, CA 95648*

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**Learning Overview:** After attending this presentation, attendees will be acquainted with information regarding the Camp Fire in, and around, Paradise, CA, which started on November 8, 2018.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sharing details of the disaster and the importance of a working relationship between forensic odontologists and the coroner's office, as well as other members of the forensic team.

On November 8, 2018, a wildfire started in rural Butte County, in northern California. The Camp Fire's name came from the place of origin: Camp Creek Road. This fire was the deadliest and most destructive wildfire in California history. Also, it was the deadliest wildfire in the United States since 1918, and ranked as the sixth-deadliest United States wildfire overall. The fire caused more than 80 civilian fatalities. It covered more than 153,000 acres and destroyed 18,804 structures. Most of the damage occurred in the first four hours.

Due to the nature of the wildfire, the victims ranged from being visibly recognizable to near-cremation. This necessitated the need for multiple forensic sciences working together. This presentation will show how several of the forensic science disciplines worked together to attain the goal of victim identification. Anthropology, pathology, odontology, fingerprints, and DNA were some of the forensic sciences represented at the morgue. Reportedly, this was the first time "Rapid DNA" was used in a mass disaster such as this.

While the fire and its victims were located in Butte County, the decision was made to send all victims to the Sacramento County Coroner's Office, which is approximately 90 miles from Paradise. Sacramento was better able to accommodate the anticipated number of potential victims. Thus, the Sacramento County Coroner's Office facilitated the victims and the workers, while Butte County remained the authorizing agency.

As many of the local dental offices were destroyed by the fire, retrieving antemortem records necessitated an alternative method. With the help of the local dental society, emails were sent out to all of the dental office in surrounding towns and cities for requests of records of reported missing persons. The records that were sent in by the surrounding dental offices were then screened by the coroner and the lead odontologist.

Early on, it was discussed that this disaster could potentially become a Disaster Mortuary Operational Response Team (DMORT) event. This led to the decision to input all data in to the WinID program. Even though this would prove to be unnecessary, it still proved to be beneficial by allowing part of the team to work remotely on the antemortem data gathering and input.

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### Wildfire, Odontology, DVI



### **G5 National Crime Information Center (NCIC) Dental Coding: Washington State Patrol's Missing/Wanted and Unidentified Persons Unit (WSP/MUPU) Cold Case Hit**

*Kyle C. Tanaka, DDS\*, Lynwood, WA 98036; Gary L. Bell, DDS, WA State Patrol Missing/Unidentified, Seattle, WA 98115*

**Learning Overview:** After attending this presentation, attendees will understand, through case examples, how the NCIC dental coding system is used to identify unidentified human remains. Additionally, attendees will gain insight into how an agency is structured to have success utilizing the NCIC system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how the NCIC dental coding system can aid in the identification of unidentified human remains. WSP/MUPU has a developed protocol to effectively utilize the NCIC dental coding system, and the unit has a long-standing working relationship with appropriately trained forensic odontologists.

On Thursday, January 30, 2019, human skeletal remains were discovered in Snohomish County, WA, by Forest Service personnel. A dental examination was completed on Friday, February 1, 2019. The following Monday, February 4, 2019, the dental information, including an NCIC-coded Unidentified Persons (UP) Digital Wide Dynamic Range (DWDR) image was forwarded to WSP/MUPU. This dental information was entered into the NCIC system on February 5, 2019, and a dental Across Matching Ranking (\$M) report was generated on February 6. That same day, the comparison report was reviewed by a trained forensic odontologist. The UP dental information was compared to the Missing Persons (MP) information in the NCIC \$M report, and a match identifying the unidentified remains was made. The Snohomish County medical examiner was notified of the identification on February 6.

A similar sequence occurred in August of 2018 when human remains were recovered in the town of Mill Creek, WA.

Again a dental examination of the remains was performed, the UP information entered into the NCIC database by the Washington State Patrol, a \$M report was generated and reviewed by a forensic odontologist, a match was confirmed, and the local medical examiner was notified.

In 2013, a review of an NCIC \$M cross match report yielded a match between an individual who was reported missing since March 31, 1992, and unidentified human remains recovered on April 29, 1992.

Washington State has state laws that designates the WSP as a central repository for all dental records for Washington States' missing/wanted and unidentified individuals. These laws (Revised Code of Washington (RCW)) include a requirement for entry of the dental information for MPs and unidentified remains into a system linked to other statewide and national systems. There are also requirements for law enforcement, coroners, or medical examiners involved in an MP investigation to collect dental information for submission to WSP/MUPU. The RCWs also include a protocol for the dental information of unidentified human remains to be submitted to the WSP/MUPU.

The protocol Washington State has established to aid in their missing/wanted and UP cases is able to utilize the NCIC dental coding system due to the WSP/MUPU's relationship with trained forensic odontologists.

This working relationship ensures that the NCIC dental coding is accurate and that the NCIC \$M cross match reports are reviewed. Law enforcement, coroners, and medical examiners can consult with the State Patrol's unit if ever aid is needed in the collection of dental data. Washington's system is a *de facto* centralized repository for the entire state.

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#### **Dental Identification, NCIC Dental Coding, Missing/Wanted/Unidentified Persons**



## G6 Exhumed Remains: A Historic United Kingdom Case Review

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**Learning Overview:** After attending this presentation, attendees will understand the importance of working in an interdisciplinary forensic team. This historic United Kingdom case of exhumed remains used an interdisciplinary approach. Attendees will also learn about the application of technology in this particular odontology case report and how this could be applied in future forensic human identification. The goals of this presentation include: (1) introducing an interdisciplinary forensic case from an odontologist's point of view; (2) recording of information at the postmortem (dental, anthropology, radiology, and pathology); (3) radiography in a mortuary (dental and full body); (4) a review of the odontology report; (5) how report writing for odontology in the United Kingdom has changed; and (6) lessons learned (from the perspective of a young odontologist).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the need for consistency in documenting dental and anthropological evidence, the importance of working with other forensic disciplines, and the application of technology in forensic reports during cases that involve human identification.

In June 2018, a major crime review team of a United Kingdom police force enlisted forensic anthropologists/archaeologists to exhume the body of an unidentified male. New evidence led to a second postmortem as the unidentified male was believed to be associated with an attempted murder in 1993. Human identification of these remains used anthropology, pathology, and odontology, along with other supporting disciplines, such as radiology. Hard tissue and dental samples were sent for DNA analysis, which subsequently confirmed his identity and confirmed that he was indeed the suspect the police were seeking.

In 1993, a man was wanted in connection with a horrific attack on a woman. The woman suffered severe head injuries, to the extent that surgeons had to rebuild her face. The suspect subsequently fled the scene using the woman's car. The police issued a number of appeals. Advice was sought from the National Missing Persons Bureau and the International Criminal Police Organization (INTERPOL). Appeals were also made on the BBC television show *Crimewatch*. No proof of life was established following the suspect's disappearance. All attempts to trace him were unsuccessful, and the case remained open.

In 1995, the body of an unidentified male was recovered from the weir of a river near the locality of the attempted murder. The unidentified body underwent a postmortem examination and was subsequently buried as a "John Doe." In 2011, the car the suspect was believed to have been driving was found in the river. The front and rear windcreens were broken. The driver's seat was pushed back and a length of wood matching the length to the foot pedals was found. No body was found in the vehicle.

In June 2018, the body of an unidentified male was exhumed by forensic anthropologists/archaeologists from Alecto Forensics. The remains were thought to be the suspect. A forensic team was assembled. A home office forensic pathologist and an accredited anthropologist examined the remains. Radiographers and police photographers assisted in the capture and collection of information. Samples for DNA analysis were taken from one molar and a section of long bone. A positive identification that matched the suspect was achieved by DNA analysis.

The forensic odontology examination was a comparison to the first odontologist's chart by Dr. Bernard (Bernie) Grant Sims at the first postmortem in January 1995. Bernie was known as the father figure of forensic odontology in the United Kingdom. His expertise was utilized in many United Kingdom major crime investigations and mass disasters such as the King's Cross Underground Fire (London, 1987), the Pam Am plane crash (Lockerbie, 1988), and the Marchioness Riverboat (London, 1989).

This case will look at the biological information that was collected during the odontology and anthropology examinations conducted during the postmortem. Reflection on how we can further improve the method of collection and recording information through the use of technology will also be explored. This odontology report showcases a simple and effective way to demonstrate antemortem and postmortem comparison dental charting.

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### Exhumed Remains, Forensic Odontology, Odontology Report



## G7 “Oscar”: The Final Chapter

Warren D. Tewes, DDS\*, Queenstown, MD 21658-1101

**Learning Overview:** After attending this presentation, attendees will understand some principles of homicide investigations that are within the usual, and beyond the usual, time and technique expectations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating action-orientated perseverance in cold case investigations. Specifically, as forensic science techniques advance in accuracy and application, the science can return to decades-old cases and bring new insight to light which, as in this case, identifies a 34-year-old unknown homicide victim, who is a 57-year-old missing person.

The identification of missing and unknown persons has been an effort since the beginning of time. It has been documented in forensic odontology from the Roman Agrippina and Lollia Paulina. The science made a quantum leap in the “Bazar de la Charite” fire disaster in Paris 1897. Dr. Oscar Amoedo coordinated colleagues in collecting and comparing antemortem/postmortem victims’ dental records for identification. In recent decades, the same is modeled in DNA comparisons.

The unknown homicide subject of this presentation was found in a galvanized trash can during the excavation of a mall site and hence nicknamed “Oscar” by scene investigators because at the time he had no name. The year was 1985 in Maryland, and evidence materials found with the remains have been analyzed repeatedly by all disciplines of forensic sciences over the decades as new techniques developed.

This case has been presented twice to the Academy in an effort to generate new leads. Serious as homicide is, this unknown was continually referenced in local media as “Oscar” and, therefore, was presented this way at the AAFS Bring Your Own Slides session in 2003. The hope was that there may be a pattern of other homicidal dispositions via galvanized trash cans. No positive outcome resulted.

With perseverance and advancing technologies, “Oscar’s” jurisdictional law enforcement agency engaged Parabon® Nanolabs to generate and publicize his phenotypic, biometric profile to facilitate new leads in a cold case. This included illustrations in *National Geographic* magazine. In 2017, these latest scientific advances on “Oscar” were again presented to the Academy for easier identification, and for encouraging young investigators to persevere by applying the newest technologies to their own cold cases.

In recent years, familial DNA research has been applied to the criminal world. In concert with law enforcement holding “Oscar’s” DNA, these newest technologies were applied. During the last week of June 2019, the Anne Arundel Police Department Maryland publicly announced their success in a press conference and revealed the identity of this long-term 1985 unknown person.

Frequently, the process of investigating missing and unidentified person homicides is to presume that the time of death is somewhat near the abduction or body recovery time, or within a year of the cycle of seasons and advancing decomposition. In this case, the 1985 autopsy report read, “. . . strands of skin were still present on the body . . .” and “Minimal remnants of soft tissue are noted in the trunk and they are not recognizable.” This suggested a reasonably recent death, and his postmortem age assessment was about 20 years.

However, he is now identified. He was a local resident, a 1961 graduate of the local high school, and according to the family, voluntarily left home in 1962. Applying postmortem time-of-death assessments to this new family history, the time of death is not early 1980s per the time of body recovery, but early 1960s. The investigation continues to determine a motive and perpetrator.

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### Cold Case, Identification, DNA



## G8 The Drowning of 26 Koreans in the Danube in Budapest

*Armin A. Farid, DMD\*, London N1 9RA, UNITED KINGDOM*

**Learning Overview:** After attending this presentation, attendees will be aware that the success of a Disaster Victim Identification (DVI) mission is founded on the unity of all national and international agencies involved; in a spirit of learning and collaboration, these agencies can provide their very best to serve society by recovering the dead and ensuring a dignified handling of their remains, as well as ensuring closure for the victims' loved ones and the survivors of any tragic event.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring the recovery and identification process, according to the International Criminal Police Organization (INTERPOL) standards, of the victims of a ship collision disaster on the Danube river in Budapest at the end of May 2019. This presentation seeks to point out that each DVI operation is unique in its nature and provides a large variety of challenges that need to be overcome in a relatively short period of time.

On May 28, 2019, at 10:00 p.m. in the evening during heavy rainfall, a sightseeing ship named “Hableány” (Mermaid), fully booked by 31 South Korean tourists, accompanied by two South Korean tour guides, with a Hungarian captain and one other crew member on board, collided against a much bigger cruise ship and sank within seven seconds. Seven tourists survived the accident and were rescued from the 10°C–15°C cold water. Consequently, seven other victims were found dead and were removed from the water under very difficult circumstances. The rescue operation, which already faced massive challenges due to the high tide, the heavy rainfall, lack of light, low water temperature, and dirty conditions, was unsuccessful in the recovery of the remaining 21 persons, who were either on the deck or inside the ship at the moment of the accident. While South Korean officials dispatched fingerprint specialists, divers, and logistical personnel, the Hungarian DVI team received its first mission to register and identify the dead bodies that were found at different spots of the Danube, the furthest 120km away. Over the following two weeks, 19 bodies were recovered from the Danube.

The mission itself stood out by the great level of cooperation between the different agencies, such as the Hungarian Counter Terror Unit, which supervised the entire operation, the Budapest Police Department, the Catastrophe Rescue Department, and the National Bureau of Investigation's DVI team. In addition to that, a very close collaboration was established with the South Korean Crime Scene Investigator (CSI) fingerprint specialists and their respective INTERPOL liaisons, the Austrian Counter Terror Unit Cobra, and foreign diving specialists.

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### DVI, Korea, Disaster

## G9 A Measurement of Morphological Features of Maxillary First Molar Crowns for Human Dental Identification

Ana M. Milheiro, PhD\*, KULeuven, Leuven B3000, BELGIUM; Cezar Capitaneanu, DMD, PhD, KULeuven, Leuven B3000, BELGIUM; Emmy Shaheem, PhD, KULeuven, Leuven B3000, BELGIUM; Steffen Fieuws, Leuven, VB, BELGIUM; Patrick W. Thevissen, PhD, KULeuven, Leuven, Vlaams-Brabant B-3000, BELGIUM

**Learning Overview:** After attending this presentation, attendees will be aware of the potential to include specific measurements of maxillary first molar crowns in human dental identification protocols.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing a method for the ranking of quantified uniqueness of specific measurements of maxillary first molar crowns and a proposition of its inclusion in protocols for human dental identification.

Improvements in oral health and the trend for minimally invasive, computer-aided dentistry makes classical protocols for human identification redundant. Morphological comparison, using 2D or 3D imaging and registration techniques, is becoming an important identification tool. This research aims to measure specific morphologic features of maxillary first molars, registered on 3D images, and to quantify their identifying power, separately and in combination.

One hundred sixty-four upper first molars were isolated from 3D digitally scanned, dental cast models and standardized dimension measurements were registered using the 3D modelling software 3-Matic Medical. Fifteen measurements were classified in four groups: tooth depth ( $n=1$ ), measurement between the cusps ( $n=6$ ), measurement between the cusps and the core of the tooth ( $n=4$ ), and angles between cusps ( $n=4$ ). All measurements were registered by the first examiner ( $n=2,460$ ). After a month, a subset ( $n=600$ ) was re-analyzed by the first examiner and by a second examiner. The distance between each subject in the subset and the subjects in the sample was quantified for each measurement to establish a Mean Potential Set (MPS) of candidate matches. The MPS, expressed as the amount of possible matches that would not exclude the correct subject in the sample, was determinant in the quantification of uniqueness of each specific measurement (univariate) and measurement combinations (multivariate). Intra-class Correlation Coefficient (ICC) was calculated to quantify inter- and intra-observer reliability. Differences and agreement between right and left measurements were evaluated.

The most unique measurement was tooth depth (ICC=0.879, MPS=17.1%), followed by the measurement between distal cusps and the measurement between the core of the tooth and the disto-palatinal cusp (ICC=0.855, MPS=19.9% and ICC=0.840, MPS=20.9%, respectively). The measurement between the mesio-buccal and disto-palatinal cusps (ICC=0.524, MPS=32.3%) was the least unique. The angles between cusps ( $n=4$ ) was the most unique measurement combination (MPS=3.911%). The intra- and inter-observer ICC (over all measures) were 0.75 and 0.78, respectively. The measurement between the mesio-palatinal and disto-buccal cusps was the only measurement revealing a statistically significant difference between the right and left side (mean difference -0.25,  $p=0.0008$ ). For this measurement, there is a clinically unimportant average discrepancy with narrow limits between the right and the left measurement. All other measurements showed a variable degree of right/left agreement.

Digital measurements of morphological features of maxillary first molars present a useful tool to integrate in human identification protocols. However, validation in forensic practice is required.

### Forensic Odontology, Human Dental Identification, 3D Morphometrics

## G10 The Uniqueness of Human Teeth: A Systematic Review and Meta-Analysis

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**Learning Overview:** After attending this presentation, attendees will be informed about current literature status regarding the proof of uniqueness of the human dentition in the forensic context. This will be outlined in a systematic review and meta-analyses of publications considering the uniqueness of human teeth and dentitions.

**Impact on the Forensic Science Community:** There is still a lack of proof regarding the uniqueness of human teeth, despite diverse research attempts. A systematic review will reveal that a small number of related studies have been conducted, mainly allied to bitemark analysis. This presentation will impact the forensic science community by demonstrating the need for future research on uniqueness of human teeth, in particular as it relates to human dental identification.

**Background:** Attempts to prove uniqueness of human teeth in a forensic context have been undertaken. Related research addressed two different forensic areas: human dental identification and human bitemark analysis. A systematic review will identify and synthesize papers considering uniqueness of human teeth and dentitions.

**Goal:** To perform a systematic literature review in order to evaluate the proof of uniqueness of human teeth and dentition morphology in a forensic context.

**Research Question:** Was uniqueness of human teeth and dentition morphology proven in a forensic context?

**Search Methods:** An electronic search was performed in six databases: MEDLINE/PubMed®, Cochrane, EMBASE®, SciELO, LILACS, and Web of Science. Additionally, reference lists of included articles and study registers were searched. There were no restrictions related to country of publication, language, or publication date.

The systematic review complied with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.<sup>1,2</sup> The review protocol was developed according to the Cochrane Guidelines for review protocols, and was registered in Prospero, the international prospective register of systematic reviews.<sup>3,4</sup>

**Selection Criteria:** To avoid bias, two reviewers independently selected articles based on titles and abstracts. Study populations, including living/deceased subjects, were considered. Review articles and pilot studies of included main studies were excluded. To check eligibility, both reviewers evaluated the full text papers independently. In case of disagreement, a consensus decision was made.

**Data Extraction and Analysis:** Data extraction and study characteristic tables have been developed. A tool for the risk of bias and paper quality assessment was developed, based on the Effective Practice and Organization of Care (EPOC) overview and Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2.<sup>5,6</sup> If data were unclear, not reported, or in a format unsuitable for the systematic review analysis, authors were contacted for further details. The articles were divided into two groups according to the area of investigation: human identification and bitemark analysis. Both groups were assessed based on the studied parameters (sample size, considered tooth/tooth part/tooth groups, registration technique for data collection, methods for data analysis, and study outcomes), and a comparison was conducted in each group and between groups.

**Results:** It was hypothesized that according to the electronic search, a small number of eligible published papers investigating uniqueness of human teeth in forensic context (approximate  $n=20$ ) will be detected. A low quality of diagnostic accuracy in these studies is expected and the extracted data comparisons will highlight that the uniqueness of human teeth in forensic context was not scientifically proven. The results will be used to address the research question.

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### Forensic Odontology, Human Dentition, Dental Morphology

## G11 Sex Estimation Using Enamel and Dentin Proportions of Human Mandibular Canines

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**Learning Overview:** After attending this presentation, attendees will understand sexual dimorphism in enamel and dentin proportions of mandibular canines and their possible role as a tool in sex determination in the future. Apart from age estimation from teeth, the use of ImageJ in sex determination from Radiovisuographs (RVGs) can also be appreciated. Attendees will learn that employing ratios in tissue proportions compensates for variation in tooth size across populations and overcomes difficulties encountered in radiographic standardization.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting a non-invasive method in sex determination using RVGs of teeth.

**Background:** Sex determination of fragmented human remains poses a challenge in archaeological excavations of mass graves, forensic anthropology, and identification of severely destroyed bodies in disaster sites.<sup>1</sup> Biological analysis of hard tissues provides nearly 100% sex identification of fragmented remains but, unfortunately, results in destruction of prime evidentiary material.<sup>2</sup> In such cases, teeth offer as non-invasive alternatives for sex determination. Dental sexual dimorphism between males and females is seen in overall size of teeth as well as in enamel and dentin proportions.<sup>3</sup> Studies on tissue proportions showed more enamel in females and greater dentin in males.<sup>4-6</sup> These studies explored tissue proportions by employing linear measurements on radiographs or area measurements on tooth sections.<sup>4-8</sup> While linear measurements are regarded as less ideal representatives of total tissue volumes, area measurements are considered accurate sex predictors but require invasive procedures.<sup>5,6</sup>

**Goals:** This study aimed to investigate sexual dimorphism in enamel and dentin proportions of human mandibular canines using both linear and area measurements by employing a non-invasive method using RVGs.

**Materials and Methods:** The study sample consisted of 85 uncalibrated RVGs (44, females and 41 males) of sound permanent mandibular canines of South Indian population in the age group of 17–35 years. All RVGs were taken earlier for diagnostic purposes and retrieved from centers with informed consent. Using ImageJ1.52a, four linear measurements (Maximum Mesiodistal Width (MMD), mesial-enamel, distal-enamel, and dentin) and three area measurements (Tooth Area (TA), Enamel Area (EA), and Pulp Area (PA)) were performed. Ratios were determined to compensate for lack of standardization of radiographs. Two ratios were determined from linear measurements (enamel/MMD and) and three ratios from area measurements (EA/TA, DA/TA, and PA/TA). Measurements were performed by a single observer. Twenty samples were randomly selected for inter- and intra-observer reliability. Data was analyzed using intra-class correlation and logistic regression analysis, and regression models were developed for sex prediction.

**Results:** Both linear and area enamel ratios were significantly higher in females than males, indicating more enamel in females. Similarly, both dentin ratios (linear and area) were greater in males indicating more dentin in males. No difference was seen in pulp ratios. A regression model using EA ratio correctly predicted sex in 69.4% of the cases, and a model using dentin linear ratio correctly predicted sex in 61.2% of the cases. The area method showed excellent intra- and inter-observer agreement. However, the linear method showed excellent intra- and poor inter-observer reliability.

**Conclusion:** Sexual dimorphism in mandibular canines is due to differences in both enamel and dentin proportions, with females showing more enamel and males more dentin. Regression models developed using enamel and dentin ratios can be utilized for sex prediction. The area method shows good reliability and sex prediction rates in comparison to the linear method.

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### Sexual Dimorphism, Mandibular Canine, Enamel/Dentin Proportions



## G12 Sexual Dimorphism in Mandibles and Permanent Mandibular Canines in a Brazilian Population: A Pilot Study

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**Learning Overview:** After attending this presentation, attendees will understand that Brazilian mandibles and permanent mandibular canines, despite the great miscegenation of its population, have a good potential for sexual diagnosis through odontometric and morphologic analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that, even with the high miscegenation of the Brazilian population, the permanent mandibular canine, and especially the mandibles, have significant degrees of sexual dimorphism, which can help in sex determination of unknown individuals when the pelvis and skull are missing.

Sex determination has a major role in the reconstruction of the biological profile of unknown individuals in a forensic context. The most reliable results for this question are obtained from morphological and metric analyses of the pelvis and skull. However, in certain cases, these bone segments are not available for analysis, which makes the forensic anthropologist capable of determinate sex from other bones. The teeth and the mandible have been widely studied as auxiliaries in sexual determination: the teeth for being resistant to high temperatures and taphonomic effects, and the mandible for being, as is the entire skull, pretty sensitive to genetic and environmental influences. Existent studies show a high sexual dimorphism in European individuals, which can be justified by the presence of a more homogenous population and a lack of significant influences of other populations. The same does not happen with South American populations, due to their distinct degrees of miscegenation.

Certainly, the use of a specific population database brings better results since the sexual dimorphism varies between the populations. Currently, there are no odontometric or morphologic standards for Brazilian individuals. Therefore, this project aims to analyze the sexual dimorphism in mandibles and permanent mandibular canines from the populations of Recife, Pernambuco, Brazil and of Vitória de Santo Antão, Pernambuco, Brazil. In Recife, 15 male mandibles, 6 female mandibles, 19 male permanent mandibular canines, and 4 female permanent mandibular canines were analyzed. In Vitória de Santo Antão, 13 male mandibles and 8 female mandibles were analyzed. In the mandibles, morphological analysis of the bone and measurement of Intercanine Distance (ID) of the mandibular canines was done with a virtual Vernier caliper. In the canines, the Mesiodistal Diameter (MD) was measured with the same caliper. All the bones and teeth are from the Contemporary Human Bones Collection from the Federal University of Pernambuco. This project was approved by the Ethics Committee CCS/UFPE n°43228015.0.0000.520.

In Recife, sexual dimorphism percentages of 16.56% for mandibles and 7.98% for right permanent mandibular canines (formula by Garn et al.) were found.<sup>1</sup> In addition, the male MD proved to be larger than the female MD; with a difference of 0.5mm to 1.0mm. In Vitória de Santo Antão, the sexual dimorphism percentage was of 6.07%. This suggests that even proximate populations have different degrees of sexual dimorphism. Other measurements were not collected due to the high rate of edentulous individuals, a problem of public health in Brazil caused by the population's lack of access to quality health. Further studies may broach the sexual determination in other teeth to avoid this problem.

This preliminary study suggests that permanent mandibular canines, and mainly mandibles, have significant sexual dimorphism and can be helpful in determining the individual's sex in a forensic context. The sexual dimorphism of more than 16% found in Recife mandibles allows an interpretation in which, contrary to previous research, an extremely mixed population may have a high degree of sexual dimorphism, but analyses with larger numbers of individuals of this population is necessary. The opposition of Recife's population to the results of other populations from South American perhaps can be explained by the populations that were mixed in the Northeast region of Brazil, primarily Portuguese (European) and Africans. This is a pilot study, with few analyzed individuals, in which future studies are needed to improve the regional population database.

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### Sexual Dimorphism, Odontometry, Mandible



## G13 Forensic Dental Identification: Using Computerized Tomographic (CT) Scans to Identify Human Remains

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**Learning Overview:** After attending this presentation, attendees will have learned how postmortem CT scans may be used in forensic odontology, in conjunction with fingerprint analysis, DNA profiling, and clinically used radiological documentation techniques such as dental periapical radiographs, bitewing films, and panoramic X-rays. CT scans may be used when these forensic methodologies are unavailable or impossible to perform on human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring how postmortem CT scans may reduce the need for jaw resections by aiding in the dental identification of human remains.

This presentation will introduce attendees to an alternative tool in aiding in the identification of a decedent, by comparing antemortem and postmortem radiographs, which is currently being used by the Office of the Chief Medical Examiner (OCME) in Maryland. This presentation will also explore the advantages and marked disadvantages of using CT scans when other methods are unavailable.

Radiography can play an important part in forensic odontology, mainly to establish identification. This may take the precise form of comparison between antemortem and postmortem radiographs. Radiographs may also be taken to determine the age of a minor victim and even help in the assessment of the sex and ethnic group.<sup>1</sup> Along with dental and DNA analysis, radiographic images are used to identify unknown individuals. This requires the securing of pre-mortem examination (e.g., CT scan or radiograph) of the suspect individual and the matching of specific anatomical details with similar postmortem studies.<sup>1</sup> Anatomical details such as the shape of the teeth, roots, supernumerary teeth, missing and present teeth, attrition, abrasion, coronal fracture, signs of bone resorption resulting from periodontal disease, bone pathology, diastemas, dental cavities, endodontic treatment, interradicular and intracoronal posts, implants, extraction socket(s), and dental prostheses can be used for identification purposes.<sup>2</sup>

Data examined will show that use of CT scans may prove remarkable because positive identification of a decedent has been accomplished by performing a CT scan on an unidentified cranium and comparing multiple landmarks and images with corresponding features in an antemortem CT scan.<sup>3</sup> Conventional CT is a useful imaging method in the process of human identification and presents innumerable advantages in this field compared to traditional radiographic projection.<sup>4</sup> CT has been utilized in the study of skulls and, in the forensic context, as an additional resource in the process of identification.

The use of CT scans may increase the quantity and quality of information involved in the death of the person examined.<sup>5</sup> Overall, there is an emphasis that the use of the CT scan may contribute significantly to forensic purposes, allowing the exploration of dental identification in cases where traditional fingerprint analysis, and classic radiographical methodologies, are not available or conclusive.

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### Computerized Tomographic (CT) Scans, Postmortem Identifications, Dental Identification

## G14 A Study of Non-Metric Dental Traits of a North Indian Population: Forensic Aspects

*Kewal Krishan, PhD\*, Panjab University, Chandigarh 160014, INDIA; Sandeep Kaur, PhD, Forensic Science Laboratory, Moahli 160059, INDIA*

**Learning Overview:** After attending this presentation, attendees will understand the usefulness of non-metric characteristics of the teeth in the identification of individuals and in forensic examinations, especially with reference to sexual dimorphism and ethnicity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the utility of morphological characteristics of the teeth in the identification of human remains, especially in a genetically disparate population.

Teeth are the hardest part of the body and can withstand even the most extreme situations, such as very high temperatures, corrosion, fire, putrefaction and decomposition, explosions and thermal trauma, as compared to the other elements of the human skeleton. Therefore, in these situations, the teeth are the most available and favored skeletal elements for the identification of the unknown human remains. Basically, there are two methods by which the teeth could be used in the identification process. The first method involves a comparison of Antemortem (AM) and Postmortem (PM) dental records of the deceased. The second method deals with making a PM dental profile in which the individualistic features of the teeth are recorded, which can ultimately help in narrowing the possible victim matches in the investigation process. The present study focuses on the variations in the morphological characteristics of the teeth, which may not only help in establishing the uniqueness and identity of the deceased in forensic examinations after comparing the AM and PM records, but also may help to estimate the sex and ethnicity/ancestry of the deceased.

The present study was conducted to examine the non-metric and morphological traits of the teeth among a North Indian population. The data were collected from a genetically disparate population of the Punjab State of North India (150 males and 150 females). Dental casts of the subjects were made by the standard procedure. These casts were studied for non-metric and morphological characteristics of the teeth. The subjects with abnormalities of the palate and lips, such as a cleft palate and cleft lip, and the subjects who were wearing partial dentures and braces were excluded from sample selection. The morphological features consist of shovel-shaped tooth, Carabelli's cusp (single-cusp, bicuspid, and multi-cusp), occlusion (normal bite and open bite), anterior cross bite, posterior cross bite, crowding, abrasion, supernumerary teeth, erosion, diastemas, crowns, peg-shaped lateral incisors, grooves, twinned, hyperdontia, abnormal crowns, crown wear, hypocones, central ridges, protostylids, distal trigonid crests, and hypoconulids. The frequencies of occurrence of these characteristic features were noted.

The statistically significant sex differences were observed in the morphological features of teeth. The most common morphological features of teeth found in males and females are Carabelli's cusp (male 90% and females 98.7%), occlusion I (male 90.7% and female 86.7%), normal bite (male 75.3% and female 72%), grooves (male 96.7% and female 73.3%), hypocone (male 98% and female 96%), central ridge (male 96.7% and female 90%), and hypoconulid (male 90.7% and females 87.3%). The least occurring morphological features of teeth found in both the sexes are shovel shaped tooth (male 23% and female 29.3%), occlusion II (male 12.7% and female 11.3%), open bite (male 4% and female 3.3%), anterior cross bite (male 2% and females 2.7%), posterior cross bite (male 5.3% and females 1.3%), crowding (male 44.7% and females 40%), supernumerary teeth (male 0.7% and female 0%), diastema (male 8.7% and female 7.3%), crowning (male 1.3% and female 0.7%), caries (male 21.3% and female 18.7%), fracture (male 4% and female 3.3%), peg-shaped lateral incisor (male 6% and female 4%), and crown wear (male 0% and female 0.7%). The study may be of great help to forensic scientists and Disaster Victim Identification (DVI) team to identify the human remains on the basis of very rare and common morphological features of the teeth. The study further cites an example for linking up certain dental traits with a particular community, especially an endogamous group.

### Forensic Odontology, Personal Identification, Non-Metric Traits



### G15 Hurricane Michael: The Role of the Forensic Odontologist

*Lisa M. Hofstad, DMD\*, Quincy, FL 32351*

**Learning Overview:** After attending this presentation, attendees will understand the role of the forensic odontologist as a responding member of the Florida Emergency Mortuary Operations Response System (FEMORS) during the Hurricane Michael mass disaster.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the significant role of mass disaster teams. Attendees will learn the mission of FEMORS and the assistance that the team was able to provide to the local district medical examiner's office during a declared disaster.

On October 10, 2018, Hurricane Michael made landfall in the Florida Panhandle as a Category 5 hurricane. Panama City, Mexico Beach, and surrounding towns suffered catastrophic destruction. Hurricane Michael caused in excess of \$25 billion dollars in damage and more than forty-five lives were lost in Florida.

The FEMORS was formed in July 2002. It is a portable morgue with a team of professionals that train annually with the mission of assisting and supporting the local district medical examiner's office during a mass incident. The state of Florida and the William Maples Center for Forensic Medicine have implemented protocols to respond to these disasters. A mission assignment was activated from the Florida Department of Health, and the FEMORS team prepared to deploy from the State Emergency Operations Center in Tallahassee on October 11, 2018. The FEMORS commander managed the deployment from the Emergency Operations Center in Tallahassee, FL. The retired FEMORS commander deployed with the activated team members to Panama City, FL.

The Disaster Portable Morgue Units (DPMU) arrived first and prepared to set up the morgue in Defuniak Springs, FL. The FEMORS team worked with the Air National Guard Search and Recovery Team. The District 14 medical examiner's office in Panama City began preparing for mass fatalities.

Despite the overwhelming destruction and devastation to areas of Panama City, Mexico Beach, and surrounding areas, the medical examiner's office in Panama City remained relatively unscathed. There was the immediate loss of power, water, and technological resources. Florida's death toll from Hurricane Michael was greater than 40 as of November 2018. The fatalities were reported in Bay, Gadsden, Franklin, Liberty, Jackson, Leon, Gulf, and Washington counties. The majority of the decedents were elderly. There were deaths as a result of storm surge, collapsed structures, crushing trees, post-storm cleanup, inability to access medical care, and homicidal/suicidal violence. The numbers continue to rise as a result of post-storm cleanup and reconstruction.

The Odontology section of FEMORS is composed of dentists, hygienists, and dental assistants. The volume of fatalities were not overwhelming for the local medical examiner's office and since the decedents were arriving slowly over several days, the entire FEMORS team was not deployed. The activated forensic odontologist worked with the Air National Guard, medical examiner, medical examiner staff, and other FEMORS team members. Some team members slept at the morgue due to generalized destruction of infrastructure, downed trees, and long transportation times to the community center facilities. During the deployment, postmortem radiographs were obtained on incoming decedents. Antemortem records were initially difficult to access due to lack of power, closed/damaged dental offices, lack of cellular phone service, etc. Upon receipt of antemortem radiographs, dental identifications were made.

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#### Mass Disaster, Medical Examiner, Odontologist



### **G16 The Incorporation of Both Community and Forensic Dentistry in the Child Abuse and Neglect Clinic in Turin, Italy**

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**Learning Overview:** After attending this presentation, attendees will possess a deeper understanding of how a dentist with experience in pediatric dentistry and a knowledge of forensic odontology can become a valuable resource in child abuse and dental neglect diagnoses and the promotion of oral health.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the added value offered by including odontologists in the multidisciplinary teams involved in investigating child abuse and in the recognition and reporting of neglect, helping to close the circle relating to child protection.

In 2002, Dr. Fulvia Negro, a pediatric physician at the "Regina Margherita" pediatric hospital in Turin, Italy, created a specific hospital clinic called "Bambi." This clinic was initially composed of pediatricians, psychologists, and social workers with the purpose of assessing and monitoring children coming from the emergency unit with a diagnosis of physical or sexual abuse. In 2013, medical examiners with a forensic background were incorporated into this unit to enhance the medicolegal evaluation and also in consideration of the judicial aspects related to the involvement with the Juvenile Court in Turin.

In 2019, a forensic odontologist with experience in community dentistry and humanitarian work was also incorporated into the "Bambi" clinic, thereby creating a full multidisciplinary approach, and introduced oral health promotion and *pro bono* dental treatments.

A specific dental form was developed to record a dental history as well as tooth and soft tissue conditions, together with any signs of physical abuse on the head and neck. Thanks to the presence of a dentist, it was possible to assess the parent's or guardian's knowledge of oral hygiene and identify any eating habits that could be harmful to the correct growth of the child. This also allowed for a dental examination to assess any potential dental neglect, which was followed by specific prescription and treatment plans, when appropriate.

Since 2002, more than 2,100 children have been seen in this unit, with physical abuse as the most distributed finding. The incorporation of a forensic odontologist was generally accepted by all the families. This allowed for a greater recognition of dental neglect cases and also enhanced the forensic evidence collection of physical signs of abuse on the mouth, face, and neck.

A specific oral health promotion program was also implemented, as the odontologist involved acted as a volunteer in community dentistry services with other volunteers.

A dentist with a forensic background and humanitarian pediatric dentistry experience can represent an additional resource in the recognition, assessment, and collection of forensic evidence in child abuse and child neglect cases. This also offers the contextual benefit of safeguarding children's oral health and well being.

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#### **Child Abuse, Forensic Odontology, Community Dentistry**

## G17 A Forensic Age Estimation From the Pulp/Tooth Area Ratio (PTR) of the Canines: A Forensic Odontological Study

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**Learning Overview:** After attending this presentation, attendees will be more aware of the crucial role of radiological methods in forensic age estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by scrutinizing the role of radiological techniques in forensic odontological identification.

Teeth are among the hardest structures of human body that remain well preserved for forensic investigations for comparatively longer periods of time as they can resist all sorts of physical, chemical, biological, and taphonomic destructions or traumatic insults. The formative, degenerative, and histological changes in human dentition after death make teeth the most reliable and significant forensic anthropological evidence for establishing identity of the deceased, such as age, sex, ethnicity, or pathological status of the deceased. For age estimations, a number of methods have been suggested by different researchers, based either on gross tooth specimens or the radiographs of the deciduous or permanent teeth, either fitted in the jaw sockets or dislocated. Radiological analysis is a non-invasive technique that has played a pivotal role in forensic age estimations of both living and dead individuals required for various purposes. The radiographic method has its own advantages, being less time-consuming, non-destructive in nature, and providing precise estimates.

The present study used radiographs of human canines recovered from human skeletal remains, exhumed from a unused well present underneath a religious structure at Ajnala, Amritsar, in April 2014.<sup>1</sup> From more than 6,000 human teeth of different types, anatomically sound canines were selected for the present investigation. Though there are some historically written versions about the identity of these non-scientifically excavated human remains, this study scrutinized the scientific validity and authenticity of various hypotheses put forth by various amateur historians and other stakeholders in an attempt to endorse or refute the written facts about the remains from their forensic anthropological examinations. The unscientific excavations have seriously challenged forensic identifications of these remains; still, there are some sound opportunities and analyses that can help to establish their identity.

The present study was conducted with the goal of estimating the age of Ajnala victims from their Pulp Tooth area Ratio (PTR) calculated from the area of the tooth surface and its pulp chamber. The morphologically intact dislocated canines were radiographed at the university health center with Siemens' digital X-ray machine and the images were stored digitally. The outline surface area of each canine and its pulp cavity was marked with the help of the computer-aided drafting program ImageJ, and their ratios were calculated. The calculated values were put into the regression equations proposed by previous researchers for PTR among different population groups.<sup>2</sup> Analysis of results and their comparisons showed that only a very few Indian studies could provide positive results when PTR values of the present study were put into their regression equations. It was found that the average age of the majority of teeth was 17–30 years or more; only 14 teeth were found to be subadults (below 14 years of age) when compared with previous studies.<sup>3</sup>

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### Forensic Odontology, Age Estimation, Pulp-to-Tooth Area Ratio

## G18 Fractalyse Software—The Analysis of the Trabecular Bone in Identification (The Continuation)

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**Learning Overview:** After attending this presentation, attendees will have acquired new information regarding the use of mandibular trabecular bone patterns to establish positive identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing a method of calculating the significance of mandibular trabecular bone patterns in arriving at a positive identification.

According to Berkeley's Orthopaedic Biomechanics Research, the trabecular bone can be classified as a porous cellular solid, consisting of an irregular 3D array of bony rods and plates, called trabeculae, which are composed of a calcified matrix. Bone marrow fills the spaces of the pores. In addition, because all free bone surfaces are covered with bone cells, bone is a living tissue that is self-healing and has the ability to adjust its morphology in response to changes in its mechanical environment, the so-called but poorly understood phenomenon of bone remodeling. As such, the mechanical complexity of this two-phase biological tissue surpasses any engineering material, making it a fascinating subject of study regardless of clinical applications.

The process of dental identification compares postmortem to antemortem data. It involves the analysis of different factors such as: the presence and absence of teeth, crown and root morphology and their interrelationships; the evaluation of the periodontal status; the type and extent of restorative, endodontic, fixed, removable, and implanted materials; tori and sinus configuration; and anomalies and pathologies of teeth and bone, as well as trabecular pattern morphology.

Few studies have been conducted on the statistical reliability of trabecular bone patterns for identification purpose. Some deal with algorithms, a mathematical expression that produces the answer to a question or the solution to a problem in a finite number of steps. Others deal with fractal analysis consisting of assigning a fractal dimension or other fractal characteristic to a dataset. The theoretical dataset, pattern, or signal extracted from a phenomenon can include natural geometric objects, sound, market fluctuations, heart rates, digital images, molecular motion, networks, etc.

Some of the studies apply fractal geometric techniques to the study of trabecular bone, to fractal analysis of radiographs by the assessment of trabecular bone structure and prediction of elastic modulus and strength, to methodological principles for fractal analysis of trabecular bone, to digital image analysis of cadaver mandibular trabecular bone patterns, to fractal dimension and lacunarity analysis of dental radiographs, to technical factors in fractal analysis of periapical radiographs, to the morphodigital study of the mandibular trabecular bone in panoramic radiographs, to fractal analysis of mandibular trabecular bone using the tile counting method, to the anatomical variations of trabecular bone structure in intraoral radiographs using fractal and particles count analyses, to the analysis of trabecular bone using site-specific fractal values calculated from cone beam Computed Tomography (CT) images, and finally to the fractal dimension of the mandibular trabecular bone measured on digital and digitized images.<sup>1-11</sup>

As a continuation of the previous research projects, *The Trabecular Bone in Identification*, *The Trabecular Bone in Identification—Algorithms and Fractal Analysis*, and *Fractalyse Software—The Analysis of the Trabecular Bone in Identification*, the current research is a continuation of the multifunctional uses of Fractalyse software, a free open source software, as an aid and noteworthy tool for trabecular bone pattern morphometric analysis and comparison.<sup>12-16</sup> Outcomes suggest that the development of a new software with a revolutionary approach to handling human trabecular jaw bone patterns for identification purposes could be possible. This approach would be especially practical in mass disaster situations involving large numbers of edentulous victims and/or with fragmented remains.

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### Forensic Odontology, Trabecular Bone Patterns, Fractal Analysis





### **G19 An Innovative Approach to the Importation and Conversion of Antemortem (AM) Dental Data to Blockchain-Protected Forensic Data for Utilization in Disaster Victim Identification (DVI)**

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**Learning Overview:** After attending this presentation, attendees will better understand the potential value of integrating blockchain technology into the field of forensic odontology. By automating the conversion and importation of a patient's dental records into a standardized AM dental database, the data can then be stored in an immutable ledger using blockchain-based architecture. This will allow the information to be securely stored and shared among DVI teams from around the world.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community, and especially the forensic odontology community, by demonstrating how blockchain technology adoption should create a universal trustworthy ecosystem for DVI teams to identify global victims more accurately and in a more timely manner than traditional methods.

Disasters are difficult to predict, and, in most cases, cannot be prevented. The number of casualties involved varies depending on the type and location of the disaster. Such disasters leave behind thousands of victims from many nations, making the process of identifying multinational victims a challenging task for the international DVI team. Collecting AM dental records from multiple nations is the key requirement in the reconciliation process with Postmortem (PM) data.

Forensic odontologists face key issues when dealing with AM dental records, which can hamper the identification process. First, the readability and quality of the recorded AM data in their original dental charts and their accessibility to the DVI team requires a solution that provides up-to-date accurate information at a minimally acceptable standardized format that can be readily understood by multiple nations. Second, dental codes are inconsistent across countries, and this requires manual conversion of dentists' codes into the standardized format for forensic odontologists used by DVI teams. This manual conversion process risks the introduction of human errors that can inadvertently introduce irreconcilable discrepancies that will hinder the reconciliation process.

This study proposes a Universal blockchain-based Dental record & Translator (UDenT) for multinational disaster victim identification. UDenT aims to automatically convert incompatibly formatted primary dental records into a unified dental record coded using standardized DVI codes such as those recommended by the International Criminal Police Organization (INTERPOL). In addition, it stores the converted dental records into an immutable distributed ledger on a blockchain-based architecture to be shared among worldwide DVI teams. This would allow for immediate access, at any time, in any place, of any victim's AM dental records in a readily understood universal format. UDenT's goal is to build the right ecosystem for DVI teams to identify global victims accurately and in a timely manner and, by creating this ecosystem, preserve their human rights along with their families.

#### **Dental Records, Disaster Victim Identification, Blockchain Technology**



## G20 The Identification of Skeletal Remains After Nearly Two Decades

Robert C. Walcott, DDS\*, Moraga, CA 94556

**Learning Overview:** After attending this presentation, attendees will understand the identification of skeletal remains with no antemortem records.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating key investigatory techniques to identify skeletal remains using search and rescue personnel with technicians, forensic odontology, anthropological human Identification (ID) laboratory, the Missing and Unidentified Persons Unit of the Department of Justice (MUPS of DOJ), news publications, DOJ DNA laboratory, and the Federal Bureau of Investigation (FBI) Combined DNA Index System (CODIS) DNA database profiles.

On March 16, 2017, a hiker discovered a skull and remains near a seasonal creek on a secluded steep hillside near the Moraga Lafayette border in California. The hiker returned to the area with Moraga police, field officers, and Contra Costa Sheriffs, including technicians equipped with dogs and metal detectors. Global Positioning System (GPS) coordinates were recorded at the site. The remains were documented at the coroner's office for determination of cause of death and identification. This office requested a forensic odontology examination. The skull, mandible, and restorations were cleaned, and the teeth were placed in their respective sockets, including a four-unit maxillary anterior fixed bridge. The dentition was charted on the DOJ Unidentified Deceased Report. A digital radiographic survey was completed utilizing DEXIS™ software with a NOMAD™ hand-held portable unit. The radiographs and completed charting were emailed to the DOJ MUPS Section in Sacramento. A search of the dental data base did not produce a comparison.

The examination revealed that there were 21 teeth present. The four third molars, maxillary left lateral incisor, and mandibular left first bicuspid were missing antemortem. Five maxillary teeth were missing postmortem. The four mandibular incisors, the right mandibular cuspid, and right first bicuspid all exhibited trauma, which may have been peri-mortem. Four of these had extensive crown fractures, both vertical and horizontal, and the other two exhibited enamel cracks. The right anterior maxillary bone above the right first bicuspid, cuspid, and lateral was missing, possibly peri-mortem, as were these teeth. Five molars had amalgam restorations and four molars had composites. A maxillary, porcelain-to-metal, four-unit fixed bridge replaced the left lateral incisor. The left central incisor had a root canal and post present. The missing mandibular left first bicuspid was also replaced by a porcelain-to-metal three-unit fixed bridge.

Two prosthodontists, six dentists, and four experienced laboratory technicians in four local cities examined the bridges. Two bay area broadcasting stations reported on this case, the *Contra Costa Times* and local newspapers published articles asking anyone with information to contact the Moraga Police Department. The Contra Costa Dental Society's monthly newsletter with photographs and radiographs published an article asking if members could assist with records. Concurrently, the coroner sent a femur bone to the California State University Chico Human Identification Laboratory. This lab provides forensic anthropology services to assist the legal system in resolving criminal cases, missing person cases, and in providing closure to families. The skeletal analysis determined that the remains were most likely a middle-aged Caucasian male approximately 70 inches in height. Subsequently, the coroner sent a femur bone to the California DOJ Bureau of Forensic Sciences, Richmond DNA laboratory. Here, extraction and purification techniques were utilized for the analyzation of the mitochondrial DNA. This lab sent their profile to CODIS, which resulted in a hit. CODIS sent this DNA type profile back to the Richmond DNA lab to be reviewed and confirmed. This positive identification was sent to DOJ MUPS, Contra Costa coroner, and the Moraga Police Department.

The identified was Richard Allen Saimons, who was reported to the Lafayette Police Department as a missing person on August 2, 1998. Saimons had lived in Lafayette and left his home on that day but never returned. He was 37 years old. On July 18, 2017, his parents were notified by the Moraga Police Department.

This case study demonstrates the application of several key techniques to identify skeletonized remains without antemortem records.

### Skeletonized, Techniques, Identification



### **G21 The Odontologist Relationship With Medicolegal Death Investigators in the Setting of the Medical Examiner's Office and Their Role in Obtaining Antemortem Dental Records for Identification**

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the importance of identification coordination and the process of obtaining antemortem dental records for comparison to postmortem radiographs for scientific identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a comprehensive overview of identification policies and protocols that can be accepted by other medical examiner offices.

The Maricopa County Medical Examiner's Office is located in Phoenix, AZ. The jurisdiction for the medical examiner's office covers more than 9,200 square miles with a population of 4.3 million people. Maricopa County is the fourth-largest and fastest-growing county, in population, in the United States. In 2018, this office received more than 10,000 reported deaths. The high case load is due to extreme heat, vast desert area, being a border state, large transient population, and winter visitors.

With nearly 700 unidentified cases admitted each year, this office implemented a comprehensive identification policy to ensure the validity of the identifications. Due to the large amount of identification cases, this office has an identification team that consists of the Identification Coordinator/Sr. Medicolegal Death Investigator, Investigator Trainer/Sr. Medicolegal Death Investigator, scene/field Medicolegal Death Investigator, odontologist, anthropologist, and advanced fingerprint technicians. The identification policy is three-fold: the decedent is either admitted as identified, as a.k.a. (tentative identification), or unidentified. The Identification Coordinator or designee assesses each case and will request an odontology consultation for cases in need of postmortem dental examinations. The consultation request is predicated by the scene investigation information. While at the scene, the Medicolegal Death Investigators attempt to locate dental information such as dental appointment cards, medications prescribed by dentists, and even toothbrushes that have a dentist's name. The Identification Coordinator or designee will subpoena the dental radiographs and written records. This is usually done with ease, but complications do occur, such as the dental office not being willing to release their records. Outreach to consular offices is essential and has been successful in obtaining dental study models and records from other countries. Additionally, there has been great success achieved by reaching out to the Arizona State Dental Association for assistance on cases that have no known dental information.

It is policy that a full mouth series of dental radiographs and dental charting is completed on all dental consultation requests. For the past five years, the odontologist has completed more than 200 cases per year. This consists of predominately identification cases, but also age estimation and bitemark cases. This office converted from conventional dental film to digital radiography approximately ten years ago and it has been highly efficient in identification cases. The image software in addition to the hand-held radiation source has been very cost effective for this county. The images are captured on a laptop, then downloaded to the county medical examiner's database. A written dental chart and an odontogram is created and also downloaded to the database.

Upon receiving the antemortem dental records, a dental identification can be made. Once the identification is completed, a formal dental report is sent to the assigned medical examiner and this report, in addition to the antemortem records, are also downloaded to the medical examiner's database. If a case remains as unidentified, these dental records are added to a local database and national database that can be compared to missing persons.

This presentation will showcase study examples of the policy in contacting private practice dentists and obtaining antemortem dental records. The presentation will also show the collaborative effort with the Arizona State Dental Association and consular offices of Mexico and Central America. The importance of having a comprehensive identification policy can be used as a guideline by other medical examiner's offices.

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#### **Dental Identification, Identification Coordinator, Medical Examiner's Office**

## G22 The Performance of Forensic Craniofacial Identification in Surgically Altered Faces

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**Learning Overview:** After attending this presentation, attendees will be acquainted with the basic principles of forensic craniofacial identification and its technical and methodological shortcomings when applied to persons whose craniofacial region was altered in the course of a maxillofacial surgical treatment. Attendees will be presented with performance rates acquired using a sizeable study sample.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an insight into state-of-the-art forensic craniofacial identification and its pitfalls related to the processing of surgically altered faces.

Forensic craniofacial identification is a set of techniques that allows forensic practitioners to establish a person's identity by comparing morphological characteristics of facial features in an unknown person (e.g., victim, perpetrator, or witness) with the physical appearance of a target or an array of candidates. Identification can be conducted in real time or off-line, based on various visual recordings via image processing (e.g., photographs, surveillance videos, medical images, etc.). Generally, image identification requires a trained professional who examines identity-coding facial features or runs computer-aided algorithms. There are fundamental differences between expert-based and computer-based image identification procedures. However, in both cases, forensic identification is known to be highly dependent on a variety of technical factors (image quality and resolution) as well as other behavioral and biological determinants (aging, pose, facial expression, postmortem modifications). Recently, surgical facial alterations performed for esthetical or other purposes (e.g., functional, trauma-related) at lower costs have become very abundant and emerged as a new covariate of craniofacial processing. However, to date there has been little scientific evidence specifying practical consequences of such artificial interventions in forensics.

The present study explores the effect of maxillofacial surgeries in forensic craniofacial identification. The tested sample consisted of 50 individuals of European ancestry aged between 17 and 44 years (average of 22.6 years) who underwent a maxillofacial surgical procedure. For each individual, pre- and post-operative image were acquired using a Cone Beam Computed Tomography (CBCT) unit and a 3D stereophotogrammetry device (Vectra M3). In addition, a control subset ( $N=500$ ) was sampled randomly from the FIDENTIS 3D Face Database ( $N\sim 3,000$  subjects; [www.fidentis.cz](http://www.fidentis.cz)).

Two basic quantitative matching techniques, landmark-based and surface-based, were employed. The landmark-based approach was based on 26 discrete facial points collected manually on 3D images, whereas the surface-based approach processed 3D meshes in their totality. For each tested individual, the post-op record was taken for a probe and superimposed, then compared in the one-to-many fashion against an array of targets composed of the individual's pre-op record and the control subset. A sum of squared distances between corresponding points (landmarks) and closest points (surfaces) were taken as the measures of dissimilarity.

The results expressed in terms of rank-1 identification rates, Receiver Operator Curves (ROCs), and likelihood ratios showed that the presence of surgical modifications raised challenges for the tested identification approaches. For the landmark approach, the accuracy rates ranged from 66% to 92%, depending on the combination of input points. The surface-based performance was likewise affected, falling to 64% of correctly matched pairs. The improvements in accuracy were observed once the mid-face and lower face regions were given a lower priority in the processing. However, in all tested instances, the acquired results fell short of the identification rates reported for similar types of face identification processing.

Similar to other scientific approaches in forensics, the primary concern for conclusions derived using craniofacial identification techniques is to be based on sound scientific principles. This requires questioning conditions, under which the employed techniques can yield accurate and reliable results. The purpose of this presentation is to raise awareness of the examined conditions to the forensic community.

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### Forensic Identification, Surgical Alterations, Craniofacial Region



### G23 The Recent Identification of a World War II Canadian Soldier: A Multidisciplinary Teamwork Approach

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**Learning Overview:** After attending this presentation, attendees will better understand the skills, knowledge, and techniques involved in the identification via forensic odontology of the skeletal remains of a Canadian soldier killed in action during World War II. This presentation will also discuss the challenges encountered when using forensic odontology for historical cases within the context of war.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sharing the ongoing responsibility and commitment by the Canadian Armed Forces (CAF) to honor its war dead. Applying current forensic science principles to historical cases allows CAF to identify recently discovered skeletal remains of Canadian soldiers and provide their families with peace of mind that their loved ones now have a proper final resting place and a headstone with their name.

The CAF, Canadian Army Dental Corps, was established on May 13, 1915. Its time-honored tradition of operationally focused dental care, both at home and abroad, has ensured that troops, past and present, have sustained the highest level of dental fitness for virtually every military operation in peace and war. As such, the present day Royal Canadian Dental Corps (RCDC) has served overseas in both the First and Second World Wars, the Korean Conflict, and in Afghanistan, as well as during peace making, peacekeeping, humanitarian, and forensic operations.

As catastrophes are of varying degrees of magnitude, Disaster Victim Identification (DVI) resources are often rapidly exhausted. Therefore, the RCDC was called upon by the Royal Canadian Mounted Police (RCMP) to aid in the DVI for catastrophes such as the Swiss Air Flight 111 crash in 1998, the earthquake in Haiti in 2010, and the First Air Flight crash 6560 in 2011. Following these disasters, the formation of the Canadian Forces Forensic Odontology Response Team (CF FORT) was created under the Canadian Forces Expeditionary Command as part of the Humanitarian Operations and Disaster Relief Contingency Plan.

The Department of National Defence's Director of History and Heritage (DHH) also relies upon the CF FORT to aid in the identification of Canadian soldiers discovered worldwide from former battlefields. DHH was founded in 1996 and is mandated to preserve and communicate Canada's military history and foster pride in a Canadian military heritage. Canada has a legal and moral obligation to care for newly discovered remains of servicemen and women from 20<sup>th</sup>-century wars.

The Casualty Identification Program was formally established in 2007 to respond to an increasing number of discovered human remains of the more than 27,000 Canadian war dead with no known grave from the First and Second World Wars and the Korean Conflict. The program's mandate is to identify these remains, whenever possible, through historic and scientific analytical techniques, and bury these remains. When the Casualty Identification Program's investigation is successful, identified human remains are buried with a name, by their unit, and in the presence of their family. Since its inception, the Casualty Identification Program has successfully identified 31 Canadians and helped with the identification of 19 foreign nationals.

On June 7, 2019, the partial remains of Sergeant John Albert Collis, discovered in January 2017, were reunited with his grave during a Government of Canada ceremony at the Commonwealth War Graves Commission's Bretteville-sur-Laize Canadian War Cemetery commemorating the 75<sup>th</sup> anniversary of D-Day and the Battle of Normandy. Sergeant Collis's immense sacrifice was recognized with his grandson, nephews, and other family members in attendance. Veterans from the Second World War, the Commander of the Canadian Army, as well as Canadian and local French dignitaries were also present to witness the interment. This positive outcome was made possible due to the strong collaborative commitment of CF FORT and the Casualty Identification Program.

#### Canada, Soldiers, Honor



## G24 Automated Identification From Dental Data (AutoIDD): A New Development in Digital Forensics

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**Learning Overview:** After attending this presentation, attendees will understand new developments in the automation of forensic identification using 3D dental images and will appreciate the functionality of this automatic program in identifying significant changes in an individual's dentition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring a novel identification system in dentistry and its application in the field of forensic dental identification and how this supports further research in the field.

**Background:** Various techniques have been proposed for automating the identification process using dental records. The use of 3D dental images has widely expanded in recent years. An efficient automatic identification program would enhance the identification process. This study proposes a novel technique to assist the forensic expert in identifying deceased individuals using an automated system, provided Antemortem (AM) records are available. This has been implemented in a newly developed AutoIDD.

**Goal:** To design a new method in Digital Forensics for accurate identification by comparing 3D dental models.

**Materials and Methods:** A new automated software was designed that uses a combination of techniques, including Iterative Closest Point (ICP) and Principal Component Analysis (PCA) for accurate identification using 3D images.

The total study sample consisted of 240 3D maxillary and mandibular dental data. The data was divided into two groups: Group A ( $n=120$ ) and Group B ( $n=120$ ).

Group A was composed of 3D-scanned orthodontic patient models from the Dundee Dental School (30 maxillary and 30 mandibular). This data was considered as AM digital data. To generate an identical sample, the dental casts of the same patients were retrieved and laser scanned to create indirect 3D dental models and considered as Postmortem (PM) digital data. This was to test the software for correct identification of all identical 3D models which validates the function of AutoIDD.

Group B consisted of 120 Intra-Oral Scans (IOS). To reconstruct a dental identification scenario, 30 maxillary and 30 mandibular IOS were obtained from 30 volunteers in the dental school and were considered as IOS-AM. After a year's interval, another 30 maxillary and 30 mandibular IOS were acquired from the same volunteers and considered as IOS-PM. This was to determine the sensitivity of AutoIDD toward any variations in an individual's dentition.

This study hypothesizes an accurate dental identification can be facilitated through the use of AutoIDD. The identification process is based on shape and alignment of the 3D dental arches, which can comprise a unique set.

A user-interface was designed to import digital dental data into Reference section (AM) and Unknown section (PM), which allows the operator to align the entire datasets to produce results—match percentage and mean distances.

**Results:** Group A Data: 30 AM and 30 PM maxillary 3D models were aligned followed by 30 AM and 30 PM mandibular 3D models. The results indicate that the system is able to correctly distinguish the matching models from the non-matches. In both the experimental studies, 100% matching results were produced.

Group B data: 30 AM and 30 PM maxillary IOS were aligned, followed by 30 AM and 30 PM mandibular IOS. The results indicate that the AutoIDD was able to accurately identify the matching AM-PM IOS from the non-matches. The match percentage ranged from 64% to 100% with a mean of 95.5% for maxillary scans and 87% to 100% with a mean of 96.5% for mandibular scans.

In summary, AutoIDD was able to demonstrate the identification of correct matches with a match percentage that clearly differentiates the matches from non-matches. This system also enables recognition of the changes in the human dentition, such as restorations and missing teeth. The match percentage is a combination of the best fit alignment and the changes identified by the system. A low match percentage may also infer that there has been significant changes to that individual's dentition, usually due to dental or orthodontic intervention.

This study presents a novel method for automatic human dental identification using 3D images and the performance of this software using digital dental data.

### Automatic Dental Identification, 3D Models, Intra-Oral Scans



### G25 Helpful Hints for a Variety of Dental Autopsy Situations and a Compilation of “Interesting” Cases

*Veronique F. Delattre, DDS\*, University of Texas School of Dentistry, Houston, TX 77054*

**Learning Overview:** After attending this presentation, attendees will have viewed a number of forensic dental identification cases, have a better understanding of methods to manage a variety of dental autopsy situations, and have viewed a selection of interesting cases. Helpful hints and methods indicated for a wide variety of dental autopsy situations, body conditions, and dental evidence will be presented through a storytelling format. Additionally, a compilation of “interesting” cases will be presented to demonstrate the types of cases a forensic dentist may be asked to consult on, and knowledge to help make the decision on whether or not to accept each “interesting” case.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information that will help provide budding forensic dentists with tools to aid their community by enhancing their readiness to serve as a consultant in dental identification cases.

The dental autopsy cases to be presented will also demonstrate the variety of “Degrees of Certainty” in dental identification cases. Positive Identification cases will be presented in which the antemortem and postmortem data match in sufficient detail to establish that they are from the same individual, within a reasonable dental probability, and there are no irreconcilable discrepancies. Insufficient Evidence cases will be viewed in which the available information, antemortem or postmortem, is insufficient to form the basis for a conclusion. Unidentified cases will be presented that demonstrate that, in some instances, there is no antemortem evidence or insufficient evidence to perform a dental comparison. Exclusion cases will demonstrate that, at times, the antemortem and postmortem data are clearly inconsistent.

Medical examiner and coroner offices often request the expertise of a forensic dental consultant to achieve the positive scientific identification that is necessary for the issuance of a death certificate. The death certificate in turn facilitates the funeral arrangements, burial or cremation of the individual, life insurance benefits, social security benefits, disposition of instructions in the decedent’s last will and testament, and matters of inheritance.

This presentation will prepare those who are in the early stages of experiencing or performing actual dental autopsies with information about situations they may encounter. Attendees will learn processes to facilitate their success as Forensic Dental Consultants. Additionally addressed during the session will be the topics of professionalism and collegiality with other forensic professionals interacted with on dental identification cases, as appropriate for the cases presented during the session.

Some of the topics to be addressed include: the recently deceased, skeletal remains, charred remains, incinerated remains, mangled remains, beaten remains, blunt force trauma remains, gunshot-to-the-head remains, decomposing remains, fragmented remains, dental evidence found at the scene that could belong to either the assailant or the victim, dental identification issues in removable prosthetics, and orthodontic evidence.

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#### **Forensic Science, Forensic Odontology, Dental Identification**



### G26 A Dental Malpractice Case Involving a Potentially Broken Dental Bur

*Cheri Lewis, DDS\*, Beverly Hills, CA 90211-3106*

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**Learning Overview:** After attending this session, attendees will have reviewed organizational requirements for an expert witness in a malpractice case. Additionally, attendees will have reviewed skills required for preparation of both depositions and expert trial testimony. Attendees will be learning a systematic approach for preparing materials in a malpractice case. Included will be preparation for a deposition and/or trial, as well as how and when a research project can assist in this preparation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reviewing the need for a detailed review of materials provided by attorneys, checking for disparities in records, and the potential use of research projects to validate opinions to be presented in both deposition and trial testimony.

This case review of a dental malpractice lawsuit involves an aspirated dental bur. The case was sent for review based on a paralegal's internet search related to aspiration cases. Guidelines for evaluation of the case will be reviewed, including, but not limited, to the information provided by the attorney. The original information provided included the patient's age and health status. Records requested and later obtained included dental radiographs of the patient along with defendant dentist treatment notes. Hospital records were ultimately provided in addition to a photograph of an aspirated surgical dental bur adjacent a ruler. The photograph provided of the aspirated dental bur was compared to the measurements of a new surgical dental bur.

The dental radiographs, in addition to the defendant dentist records, will be discussed, as will alternate treatments that legally should have been presented to the patient. The technique utilized to review the dental records from the defendant dentist, including his radiographs, his depositions, and hospital records, will be discussed. Inconsistencies within the records provided will be brought forth. Safety measures that were available and should have been provided to protect the patient's airway from the aspiration of the dental bur will be discussed.

Ultimately, a research project was conducted to clarify the standard of care among oral surgeons regarding patient protection against aspiration of foreign objects during surgical dental extraction procedures. The technique for setting up this research project will be reviewed, and the findings and value will be discussed. Preparation for expert depositions will be reviewed, including disclosures of exhibits required. The need to provide all exhibits at the time of the deposition will be reviewed.

Also discussed will be the need for the expert to review the case with the plaintiff attorney, the expert's responsibility for assisting in setting forth questions for the plaintiff attorney, the type of questions that should be provided for the plaintiff attorney to be used with the defendant dentist and his experts at the time of their depositions and later in the courtroom, and questions relating to the standard of care for oral surgeons providing surgical extractions and the requirements of a general dentist to adhere to the same standards.

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#### **Malpractice, Dental, Research**



## G27 Thresholding Adulthood: What Are We Doing?

James F. Goodrich, FFOMP\*, Cambridge, Waikato 3496, NEW ZEALAND

**Learning Overview:** The goal of this presentation is to describe the current application of dental age estimation procedures with respect to thresholding adulthood in many parts of the world and provide estimates of the number of errors made. The forensic odontologist engaged in providing opinion to any authority around thresholding adulthood should be aware of the limitations of the science that they employ and the implications to the individual concerned.

**Impact on the Forensic Science Community:** The role of forensic scientists in advising policy makers is important to the outcome of the application of that branch of science. If mistakes are being made in the area of thresholding of adulthood, solutions need to be discussed and efforts made to reduce or eliminate those mistakes. This presentation will impact the forensic science community by informing attendees of some of the challenges faced in this area of science, and some possible solutions to help them maintain a role as a responsible forensic practitioner.

The idea of an age at which adulthood is reached is a common notion worldwide. This chronological marker varies, and has different implications, in different parts of the world. This threshold age of adulthood is almost invariably an inflexible and meaningful border between being a child and being considered by the law, as well as by society, as an adult. The threshold age itself may well be viewed as an arbitrary value, given the variability in human maturation, but it is nonetheless a strict and binary judge between childhood and adulthood. It is a breach of the human rights of any child to be treated under the law incorrectly as an adult.

In many cases, the chronological age of an individual is not known. In such cases, the law often requires an age to be ascribed to these individuals, along with the rights and responsibilities of that age group. This process often occurs when undocumented people are crossing borders. Many methods have been employed to aid with this determination of age, all of which are based on a system of estimation.

Dental maturity is widely regarded as one of the most reliable indicators of chronological age estimation.<sup>1</sup> Unfortunately, the accuracy of dental age estimation varies with the age and stage of the individual concerned. As a general rule, dental aging methods tend to be more accurate the younger the individual in question is, and thresholding adulthood, if that age is 18 years, is difficult.

Dental age estimation around 18 years of age tends to rely on the development of the third molar teeth. Famously, in 1993 Harry Mincer et al. published the results of a study of 823 individuals and he found that "... the association between chronological age and the formation of the third molar is, at best, moderate," and that with intermediate indicators only, he described it as a "coin-toss" if the individual had reached 18 years of age.<sup>2</sup> Since that time, many researchers have strived to improve on our ability to reliably threshold a given individual as an 18-year-old using dental maturation.

Forensic odontologists have a responsibility to examine the methods they are using, and how the information that they are providing is being used. As a group, we should always be considering our role in any forum for which we provide information. In this case, the question that may need to be discussed is "despite us being the best at age estimation around thresholding adulthood, what level of inaccuracy is acceptable to us before we shouldn't be doing it at all?" The justification that we are not the judge, and we are simply supplying information to a decision maker, may or may not be ethically satisfying.

Simply put, despite best intentions and skillful application of the known science, because of the limitations of our current methods, forensic odontologists may be involved in a process that sometimes wrongly thresholds a child as an adult and visa versa.

Our role as forensic odontologists also includes educating policy makers around the functional application of our science. If there is a problem with the way our expert opinion is being used, then we should be actively engaged in providing a solution.

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### Age Assessment, Thresholding Adulthood, Human Rights

## G28 Method Differences, Population Differences, or Examiner Differences: Which Affects the Age Estimation the Most?

Sakher J. AlQahtani, PhD\*, College of Dentistry, Riyadh 11545, SAUDI ARABIA

**Learning Overview:** After attending this presentation, attendees will be able to take a more critical look at the concepts of accuracy and precision as they relate to dental age estimation. In addition, attendees will be able to understand that method selection, utilization of population-specific databases, and failure to calibrate examiners have different effects on the accuracy in reporting of age estimation interval.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of not only applying the correct testing methodology on dental age assessment technique, but this presentation will also encourage the community to take a more critical look at comparing the effects of examiner calibration versus the use of population-specific databases in the reporting of an accurate age estimation interval.

Although dental age assessment methodologies have been extensively researched, tested on different populations, and modified throughout the years, there is still contradictory evidence as to what are the key parameters to consider when selecting the appropriate reference database to use on an individual case. One area of concern is that there is a belief that there are significant differences between different geographic and ancestral populations, and it is critical that population-specific databases be utilized by age assessment techniques. In the past, differences expressed by researchers between test groups have universally been interpreted as proof that population differences exist. Unfortunately, most researchers have failed to look critically at the possibility that other factors, such as examiner calibration and bias, and not population or sex differences, may have had a greater influence on these differences.

The goal of this study was to compare the interpretation of results and particularly differences between three dental age estimation methods, Moorrees, Fanning, and Hunt's stages of dental development, Demirjian's dental development scores, and the London Atlas of Human Tooth Development and Eruption, to determine the sources of variances when taking into account subjectivity and user bias.<sup>1-3</sup> An additional goal was to take a more critical look at the concepts of accuracy and precision as they relate to dental age estimation when a technique is tested.

**Conclusion:** Differences in most published research between dental age techniques and population differences actually falsely attribute these differences to population variance when they should be attributed to the testing methodology of that technique. Measures of performance, study design, and reporting of results when a technique of dental age assessment is being tested need to be revised, especially when measuring population variance.

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### Dental Age Estimation, Accuracy, Population Variance

## G29 Forensic Age Estimation Based on the London Atlas of Human Tooth Development and Eruption Assessment in a Population With Systemic Disorders: A Pilot Study

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**Learning Overview:** After attending this presentation, attendees will understand why using the London Atlas of Human Tooth Development and Eruption on panoramic radiographs is of limited value for age estimation in children and subadults with special needs, such as with systemic disorders.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing why using the London Atlas is of limited value for age estimation in a special needs population, for example, those with systemic disorders.

**Introduction:** The forensic estimation of chronological age has played an increasingly important role, not only in the identification of human remains, but also in living individuals due to the phenomenon of immigration and sexual abuse of undocumented trafficked children.

**Goal:** The goal of this research was to validate the use of the London Atlas, an atlas already used in the normal population, in estimating chronological age in a population with special needs and/or with systemic disorders.

**Materials and Methods:** A sample of 163 orthopantomograms from two independent medical institutions were collected from 133 patients, between 4 and 23 years of age, with systemic disorders in the appointment of patients with special needs. The orthopantomograms were divided into two groups, 95 from patients with systemic pathologies that have repercussions on dental development and 68 with systemic pathologies without dental repercussions. Dental ages were estimated by the London Atlas using the left side, then independently using the right side of the maxilla. The intra- and inter-observer agreements were evaluated. The difference between the dental age estimates and the chronological age and its absolute value were computed and analyzed.

**Results/Discussion:** Statistically significant differences were obtained between estimates and chronological age, revealing a general prevalence for underestimation; except for ages less than 12 years. Nevertheless, the underestimation in individuals less than 16 years of age was not significant (with an average of less than one month), while the underestimation was quite significant for patients at least 16 years of age (with an average over 26 months). Furthermore, for those patients with systemic diseases with dental repercussions, a greater error in underestimation was obtained, which indicates that the midpoint values should be reassessed in patients with Down's syndrome, chromosomal alterations, syndromes, and central nervous system disorders.

**Conclusions:** Hence, this Atlas can be potentially used as a tool for age estimation, but we suggest further studies with larger samples to create adequate atlases for all the required scenarios, in particular, diagrams for patients with special needs development who are more than 12 years old and, specifically, for those more than 16 years of age.

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### Forensic Age Estimation, London Atlas, Systemic Disorders

## G30 Dental Age and Odds Probability at the 18-Year Threshold

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**Learning Overview:** After attending this presentation, attendees will understand different strategies for presenting data in forensic age estimation reports based on expert witness reports in the United Kingdom Immigration Court in relation to the 18-year threshold.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how the former legal threshold of Preponderance of the Evidence (which in Europe is referred to as "Balance of Probability" (> or < 50%)) has transmogrified into 100% Certainty as the level of proof. The Minimum Value Threshold (beyond reasonable doubt) is the level of proof recommended in Europe as promoted by the European Commission.<sup>1</sup>

**Issues for Consideration:** Use of teeth as an indicator of age is the most reliable method for estimating chronological age using human biological growth markers based on Normal distribution summary statistics.<sup>2</sup> Arising from this is Simple Probability (SP), which provides a neat technique to estimating the probability that a subject is more than 18 years of age.<sup>3</sup>

There are four possible approaches to expressing uncertainty in relation to the 18-year threshold and the lower left third molar.

Simple probability—this requires the full raw dataset.<sup>4</sup> Results for over 18 years old are: United Kingdom-Whites,  $p=0.7920$ ; Iran,  $p=0.9483$ ; and for Southern Chinese,  $p=0.9991$ .

Odds—this also requires the full raw dataset.<sup>5</sup> *Specificity* and *Sensitivity* are calculated. This enables estimation of the Odds. In this context, the Odds of a subject with a mature 3<sup>rd</sup> molar for United Kingdom subjects is (approximately) 1:4; for Iranian subjects, 9,483:1; and for Southern Chinese subjects, the Odds are 9,991:1.

Likelihood Ratio (LR)—this also requires the full raw dataset.<sup>6</sup> For United Kingdom subjects, this is given by  $0.792/0.208=3.82$ . This is weak support for the view that the subject is more than 18 years old. For Iranian subjects, the LR is given by  $0.9483/0.0517$ , which gives the LR as 18.34. This is moderate evidence to support the contention that an Iranian subject is more than 18 years old. For Southern Chinese subjects, the LR is given by  $0.9991/0.0009$ , which is 1110.1. This is compelling evidence that a Southern Chinese subject with a mature 3<sup>rd</sup> molar is more than 18 years of age.

Minimum Threshold Value (MTV)—this comes from European Commission Guidelines.<sup>1,7</sup> It is a robust marker for age and provides a 100% probability that a subject is below the minimum age of the Tooth Development Stage used for assessment. The MTV has the advantage that values from summary statistics in published papers can be used to make the assessments. It is not necessary to have access to the raw data.

**Concluding Remarks:** The MTV is an unambiguous technique for indicating whether or not a subject is under (or over) the 18-year threshold.

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3. Kasper K.A., Austin D., Kvanli A.H., Rios T.R., Senn D.R. Reliability of third molar development for age estimation in a Texas population: A comparison study. *Journal of Forensic Sciences*. 2009; 54(3): 651-657. doi:10.1111/j.1556-4029.2009.01031.x.
4. Draft D., Lucas V.S., McDonald F., Andiappan M., Roberts G. Expressing uncertainty in Dental Age Estimation: A comparison between two methods of calculating the 'average standard deviation'. *Journal of Forensic Sciences* 2019; April: 1-4 doi 10.1111/1556-4029.14049.
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### Dental Age Estimation, Minimum Threshold Value, Odds Values and Likelihood Ratio



## G31 Calculating the Standard Deviation: An Innovative Approach for Utilizing Historical Databases

Sakher J. AlQahtani, PhD\*, College of Dentistry, Riyadh 11545, SAUDI ARABIA; Kenneth W. Aschheim, DDS\*, New York, NY 10065

**Learning Overview:** After attending this presentation, attendees will understand how a new, proposed method in the calculation of standard deviation combining historical databases with modern populations' databases in the reporting of a dental age assessment can greatly improve the reporting of the age interval.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science and legal community by improving the accuracy of dental age assessment techniques by combining traditional methods of calculating an age estimation interval using a new, proposed method. A step-by-step process of calculating standard deviation using three different methodologies will be detailed.

Age estimation remains one of the more complicated and difficult aspects of the human biological profile to assess. In addition, when used on living individuals, a dental age assessment has the potential to greatly affect their future and, if done improperly, can potentially violate their human rights. Given the risk involved with flawed reporting, concerns raised over the accuracy of the age estimation interval are justifiable, and scrutiny over its reporting understandable. Finding the most accurate method of calculating the standard deviation for a dental age assessment technique is essential to allow for a precise range calculation, and that the technique will apply an appropriate age interval to an individual. The calculation of a standard deviation is particularly complicated because not only there is variability in the historic dental developmental tables but variability in the rate of development of each of an individual's tooth buds as well. Numerous factors have been hypothesized to influence the rate of dental tooth bud development; however, for many of these factors, there has been no appropriate studies to verify that the effect and, if it exists, its magnitude.

Because of the complexity of these calculations as well as the lack of consensus on calculating of the standard deviation, many dental age estimation techniques, each with its own standard deviation calculation methodology, exist. In this study, different traditional methodologies of calculating a standard deviation, as well as a new innovative approach utilizing both a traditional database as well as a secondary population-specific database, was tested on a Saudi population using three dental age estimation techniques: Moorrees, Fanning, and Hunt's stages, Cameriere's open apices formula, and the London Atlas of tooth development.<sup>1-3</sup> A step-by-step process of calculating standard deviation using these different methodologies will be detailed, and the test results will be explained.

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### Dental Age Estimation, Accuracy, Standard Deviation



## G32 Third Molar Development in Caucasian and Chinese Populations and Its Implication in Dental Age Estimation

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**Learning Overview:** After attending this presentation, attendees will be able to appreciate the variations observed in the development of third molars between two distinct ethnic populations. This will be presented in the form of a research study that sought to compare each stage of development of third molars in a large sample of Caucasian and Chinese populations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of ethnic variations in the development of third molars. Attendees will learn how these variations could influence the accuracy of estimated age, particularly in the 18-year-old threshold.

Evaluation of dental development is useful in forensic dentistry to estimate the age of subjects without authentic birth documentation or those involved in falsified age claims. The 18-year-old threshold is important in forensic age estimation as in most countries, this age differentiates child from an adult. For this purpose, investigators rely on the development of third molars as they are the most reliable teeth for age estimation at this threshold. Variation in dental maturation has been shown in ethnically different populations.

This study aimed to evaluate the dental development of third molars in Caucasian and Chinese populations. Dental panoramic radiographs of 1,670 subjects constituting 898 females and 772 males aged 14 to 23 years were obtained from the archives of teaching hospitals in London and Hong Kong representing Caucasian and Chinese populations, respectively. The maxillary and mandibular right third molars were scored according to Demirjian's classification for the root development stages (E to H). The number (n), mean age ( $\bar{x}$ ), and Standard Deviation (SD) of the mean age was derived for each tooth development stage for females and males separately in both populations. Statistical significance was set at  $p < 0.05$  and an independent sample  $t$ -test was used to compare the mean age for each stage of development between the Caucasian and Chinese populations. In all the stages, the Caucasians were more dentally advanced than the Chinese. The mean age difference between Chinese and Caucasian females ranged from 1.18 years to 3.50 years, and in males it ranged from 0.63 years to 2.71 years ( $p < 0.05$ ). It is concluded that the Caucasian subjects demonstrated significantly advanced dental maturation of both maxillary and mandibular third molars in both sexes compared to the Chinese subjects and this emphasises the utilization of appropriate population-specific data for age estimation.

### Third Molar, Dental Maturity, Age Estimation

## G33 Minimum Values for Mandibular Maturity Markers

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**Learning Overview:** After attending this presentation, attendees will understand that for clinical dental age estimation in Europe, there is a prevailing view that only the minimum value of age at assessment reference data is the legally acceptable threshold in Europe. This applies at all ages, but particularly at the 18-year threshold.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that for the European Union, the legal burden of proof is 100% certainty for determining whether or not a subject is below or above 18 years of age. This has considerable impact because in the United Kingdom and European Courts, the civil burden of proof of "greater or less than 50%" no longer applies. In the United States, this is called the "preponderance of the evidence."

**Introduction:** The use of human biological growth markers to help estimate chronological age has undergone a shift from the use of normal distribution statistics with the "preponderance of the evidence" to the rigorous use of age marker-based assessment criteria using the minimum value (0th%ile) of an age marker as the criterion.<sup>1,2</sup> The purpose of this report is to review published data to determine if the variability of the age at assessment for the minimum value of Root Pulp Visibility (RPV) discriminates between <18 years and >18 years.

**Materials and Methods:** The data used as the starting point are from a previously presented paper.<sup>3</sup>

The summary statistics for males only in decimal years are shown in Table 1.

	n-tds	$\bar{x}$ -tds	sd-tds	Minimum	25th%ile-	50th%ile	75%ile	Maximum
RPV-Am	213	21.27	2.05	17.16	19.67	20.91	22.80	25.49
RPV-Bm	272	22.61	2.15	17.71	21.06	22.77	24.38	25.93
RPV-Cm	66	23.34	1.98	18.16	22.13	23.76	25.05	25.96
RPV-Dm	9	23.46	1.67	20.19	22.41	23.47	24.87	25.19

It can be seen that the minimum values for RPV-Am and RPV-Bm are below the 18 year threshold whereas the minimum values for RPV-Cm and RPV-Dm are above the 18 year threshold.

This is offered as compelling evidence that the mandibular maturity marker of RPV discriminates between a juvenile and an adult.

This was rejected by the United Kingdom Court of Immigration as it was "too novel" and needed confirmation from other studies. Further support has been provided by studies in Germany-2, Portugal, Western China, the United Kingdom, and Malta.

**Results:** The minimum values for RPV-Cm and RPV-Dm for the seven countries are shown in Table 2 (decimal years).

Stage	Germany -1	Portugal	United Kingdom	Germany-2	Northern China	Malta
RPV-Cm	24.70	18.80	18.58	22.10	24.68	18.23
RPV-Dm	25.20	21.20	22.45	25.10	27.66	23.99

**Discussion:** These multi-ethnic data from several disparate countries, but using the same methods, provide similar results. All the studies provide satisfactory assessments of between assessor and within assessor performance.

**Conclusion:** RPV stages C and D at the minimum value demonstrates the validity of using this age-related growth marker to determine in legal terms whether a subject is a juvenile or an adult.

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### Mandibular Maturity Markers, Root Pulp Visibility, Minimum Age Threshold



## G34 Demirjian 2.0

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**Learning Overview:** After attending this presentation, attendees will have gained a basic understanding of the historical significance of the original Demirjian dental age estimation studies and their impact on current dental age estimation cases routinely performed by forensic odontologists. Attendees will also learn about the current status of the historical Demirjian data collection and its availability for future research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reviewing the significance of the historical Demirjian dental age estimation studies and discussing the limitations of the original study and calculations and how the original data will be managed and utilized in new modern studies.

The forensic odontologist is often called upon by agencies to estimate the age of both living and deceased individuals. The forensic odontologist often applies published studies that provide a method to analyze the dentition of an individual as well as a mathematical formula and/or a table of reference data to calculate the estimated age.

Some of the more significant historical studies for dental age estimation were conducted by Demirjian, et. al, in the 1970s. Dr. Demirjian conducted several longitudinal studies of children and adolescents over the span of 20 years utilizing dental models and radiographs of the developing dentition along with radiographs of the hand and wrist. Their data was published in several studies, which set the standard for future age estimations cases.

One of the major critiques of the original study as used for dental age estimation was the calculation and utilization of a maturity score index. The method of calculation has never been fully understood and in practical use was found to consistently underestimate the age of the studied individual. Another critique is the reporting of the age interval in a graphical format in percentiles making it difficult to accurately report the age and associated interval. The current common accepted method of reporting the age interval is in standard deviations.

With Dr. Arto Demirjian's assistance, the archives of the original data have been located. Dr. Demirjian has graciously made the original data available through the University of Montreal. The data is currently being digitized, and it is the intention of the University of Montreal to make the data available to qualified researchers for future studies.

This study in conjunction with the University of Montreal also intends to redo the original studies and provide forensic odontologists and other disciplines with a modernized study for dental age estimation cases, including standard deviations to report the age interval.

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### Age Assessment, Demirjian, Standard Deviation



### G35 A Comparison of Dental Age Estimations From Two Radiographic Methods of Metric Analysis in North Indian Young Adults

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**Learning Overview:** After attending this presentation, attendees will better understand the comparison between the efficiency and accuracy of two methods for age estimation based on the same factor/principle but with different forms of measurement of the pulp chamber area. This presentation will also apprise attendees of the population-specific variations present in the reduction of the pulp chamber with age.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring the variations in the deposition of secondary dentine, believed to be genetic-based and population-specific. This would also encourage scientists to formulate a standard formula/method suitable for all populations, which is a need of the hour due to the admixture of races and globalization.

Age estimation is one of the important components of the biological profile of an individual. Numerous dental age estimation methods have been suggested by different researchers, based either on gross tooth specimens or the radiographs of the deciduous or permanent teeth, either fitted in the jaw sockets or dislodged ones. Radiological analysis is a non-invasive technique that has played a very crucial role in forensic age estimations of both living and dead individuals required for various purposes. The radiographic method has its own advantages, being less time consuming, non-destructive in nature, and providing precise estimates. Forensic age estimation is no longer limited to the identification of the dead or skeletonized remains, but has been commonly warranted by law enforcement agencies for age estimation of even living individuals in cases such as age-related frauds, crimes to avail certain social or legal benefits, etc. Teeth are the ideal evidence in most of such forensic scenarios due to their slow rate of decomposition and higher resistance to morphological or taphonomic degradations compared to other osseous parts of human body.<sup>1</sup> They are the reservoirs of biological information of the individuals, being least affected by external factors. Radiographical assessment of teeth, a non-invasive technique, further augments their significance for age evaluation in the living individuals as well.<sup>2</sup> Estimating age in children is an easier task since the development stages follow more or less a definite pattern, unlike adults, whose teeth undergo degenerative changes at a much slower rate than the formation stage and depict wide variations due to environmental, nutritional, and genetic factors.

This present study is based on radiographic quantification of the intrinsic changes occurring in the teeth in the form of deposition of secondary dentin in the pulp chamber with advancing age, thus leading to area reduction in the latter.<sup>3</sup> Such variations in pulp width is measured using two radio-metric methods, namely the Tooth Coronal Index (TCI) and Pulp Chamber Tooth Height Ratio (PCTHR). Most of the studies conducted for estimating age using these methods have indicated the need for population-specific standards for more accuracy.<sup>4,5</sup> This study proposes estimating the age of 300 adults (aged 21 to 50 years) belonging to a North Indian population, comprised of individuals belonging to Himachal Pradesh, Punjab, Haryana, and Chandigarh. No such study has been conducted on this population, per research.

The digital panoramic radiographs of the subjects were collected after their written informed consent and ethical clearance from Institutional Ethics Committee of the Government Medical College and Hospital (GMCH), Chandigarh. Collected images were analyzed using ImageJ software. The goal of the present study is to compare the validity and precision of the above-mentioned two radiographic methods in estimating the age of North Indian individuals. The preliminary results based on 102 samples indicated the error range of the TCI and PCTHR methods in age estimation as  $\pm 5$  and  $\pm 6$  years, respectively. The conclusive analysis of the present study is underway and the results and their interpretations, along with the comparative efficiency of these two dental age estimation methods, will be presented.

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#### Forensic Age Estimation, Panoramic Radiographs, North Indian Population

## G36 Dental Development in a London Population of Diverse Ethnicity

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**Learning Overview:** After attending this presentation, attendees will understand that ethnic-specific reference data are essential for dental age estimation, especially around the 18-year-old threshold.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing ethnic differences in third molar development between Black British and White British subjects. The attainment of Demirjian tooth development stages in third molars occurs earlier in Black British subjects compared with White British subjects.<sup>1</sup>

The aim of the research was to test the null hypothesis that there is no ethnic difference in the timing of tooth development in Black British and White British children and young adults.

This study (IRAS Project ID 239922) is relevant because ethnic or ancestral differences in dental maturation have been inadequately studied. Data was collected from digitized Dental Panoramic Tomographs (DPTs) from the archives of a teaching hospital. This convenience sample compared dental maturation of Black British and White British ethnic groups residing in and around London. The main inclusion criterion was a self-assigned ethnicity in the hospital records. All those reporting Black ethnicity were included in the Black British group. For both Black British and White British ethnicities, a DPT for up to 50 male and 50 female subjects in half-year age bands in the age range of 11 to 24 years was examined. The current sample totals 3,675 subjects. The Anglo-Canadian eight-stage scoring system was used.<sup>1</sup> Datasets for females and males of the two ethnic groups were compared. Nearly all stages of third molars developed earlier in the Black British group compared to the White British group, the difference being greater in females. For Demirjian Stages D, E, F, and G of all four third molars, with the exception of Stage D in the right-sided third molars in females, Student's *t*-tests demonstrated a significant difference ( $p < 0.03$ ).

The table below shows results for the lower left third molar (LL8) Stages A-H<sup>1</sup> in males aged 11-24 years.

Results for lower left third molar (LL8) Stages A-H for males (Ages in decimal years)												
TDS	Black British					Difference between		White British				
	<i>n</i>	Mean	SD	Min	Max	Means	<i>p</i> value	<i>n</i>	Mean	SD	Min	Max
LL8A	0							24	12.41	1.21	11.01	15.09
LL8B	3	13.20	0.45	12.71	13.62	-0.40	0.5570	62	12.81	1.15	11.05	16.70
LL8C	30	13.09	1.19	11.61	15.69	0.37	0.1739	149	13.47	1.40	11.05	17.87
LL8D	47	13.34	1.29	11.15	16.36	1.10	0.0001	65	14.44	1.54	11.74	19.28
LL8E	65	14.38	1.52	11.44	19.53	1.45	<0.0001	131	15.83	1.78	11.50	21.57
LL8F	48	16.11	1.14	14.19	18.73	1.32	<0.0001	87	17.42	1.51	13.15	20.83
LL8G	46	17.52	1.36	14.93	20.71	1.28	<0.0001	80	18.80	1.50	15.76	23.41
LL8H	74			14.13	20.69		<0.0001	330			16.28	23.39

Ages compared using Student's *t*-test for Stages A-G and Mann Whitney Test for Stage H (censored)

Censoring the ages at 20.71 years for Black British and 23.41 years for White British (i.e., the maximum ages for LL8 at Stage G), a Mann Whitney test showed significant difference ( $p < 0.0001$ ) between the two ethnic groups for Stage H of the LL8.<sup>2</sup>

The following table shows percentages of all assessable third molars at Stages A-H in 17- and 18-year-old males, illustrating earlier development in Black British compared to White British subjects.

TDS	TDS A-H of all assessable third molars (%)			
	18-year-old males		17-year-old males	
	Black British	White British	Black British	White British
A	0	0	0	0
B	0	0	0	0
C	0	0	0	1
D	0	0	0	4
E	4	13	3	20
F	10	26	21	32
G	38	21	38	26
H	48	40	38	17
Total	100	100	100	100

In conclusion, differences between Black British and White British ethnic groups from a London population indicate that ethnically appropriate reference datasets are essential for age assessment throughout childhood and young adulthood in skeletal remains as well as the living but may be particularly important when decision-making regarding the 18-year-old threshold.

The data demonstrate that there are highly significant differences in dental development between Black and White ethnic groups in a United Kingdom population.

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- <sup>1</sup> Demirjian A., Goldstein H., Tanner J.M. 1973. *Human Biology* 45:211-27.
- <sup>2</sup> Roberts G.J., McDonald F., Andiappan M., Lucas V.S. 2015. *Journal of Forensic and Legal Medicine* 36: 177-184.

**Dental Age Estimation, Ethnicity, 18-Year-Old Threshold**



## G37 An Analysis of a Dental Prosthesis Technique in Korean War Casualties

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**Learning Overview:** After attending this presentation, attendees will understand the differences in materials used in dental prosthetic manufacture between Korean and Chinese dentistry in the 1950s.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by employing an unusual attempt to analyze the materials used in dental prosthesis through Laser-Induced Breakdown Spectroscopy (LIBS).

During three-year Korean War (1950-1953), numerous soldiers were killed or went missing. In 2007, the Korean government established a special organization, the Ministry of National Defense Agency for KIA (Killed in Action) Recovery and Identification (MAKRI), to recover and identify the remains of unaccounted-for casualties. However, due to the fact that most of casualties are of Asian origin, determining the nationality based on the DNA haplotype is sometimes very challenging. Therefore, various studies have been attempted to overcome those limitations. Analysis of prosthetic materials by nation is one of those efforts. In addition, due to the lack of previous studies, it was important to compare the results and accumulate a database.

The 35 dental remains with dental prosthesis, determined to be those of Korean and Chinese service members from the Korean War collected at the MAKRI Central Identification Laboratory (CIL) during the period from 2015 to June 2019, were assessed. All samples were analyzed using LIBS equipment to contrast atomic emission peak results by nation and prosthesis type.

In the results of LIBS, it was observed that metal crowns confirmed specific atomic emissions, such as Al, Mg, Zn, Fe, and Cu, in Korean and Chinese prosthesis. All silver-colored crowns were confirmed to contain specific atomic emissions, such as Ni, Al, Cu, Ag, Cu. Furthermore, it was observed that there were similar spectral peak values on Ni, Au and Ag. However, silver-colored crowns were not observed among the Chinese dental remains. It seems that this dental technique is not shared in both countries. This study indicates that the results of dental prosthesis comparison has considerable utility in cases of the same Asian ancestry in which is difficult to estimate the ancestry.

In conclusion, Korea's dental technique was very similar to the Chinese dental technique, such as full-coverage crowns and full-coverage crowns with a cantilever pontic, but was not similar as regards silver-colored crowns at the time of Korean War.

### Korean War, Nationality Determination, Dental Restoration



### G38 The Advantages and Limitations of Various Dental Age Estimation Methods in Forensic Odontology: A Systematic Review

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**Learning Overview:** After attending this presentation, attendees will be informed about the utility and shortcomings of various age estimation methods used by forensic anthropologists.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees about the use of different age estimation methods in different contexts and their usefulness and limitations.

Teeth, along with associated oral structures, play an important role in the identification of an individual as they are considered storehouses of invaluable information for biological, physical, chemical, and medical sciences and may provide vital information about the identity, provenance, migration history, dietary status, trauma, etc. Dental structures are the hardest and most well-protected structures of the human body and can resist decompositions, incinerations, and other degradations.

Age is an important aspect of the biological profile that is estimated from human skeletal remains retrieved from forensic scenarios, such as disasters, accidents, or crime scenes. Age is also an important factor in clinical practice, research endeavors and courts of law. In addition to some administrative and judicial reasons, forensic age estimations have become vital for checking illegal population migrations, fraudulent old-age pensions, and asylum seekers. The lifetime biological changes and events occurring in the human body are impregnated in the hard tissues (bones and teeth), which are directly related with the biological age of an individual. Statistically significant correlation exists between the chronological age and the growth and development status of an individual.

Forensic odontology is playing a very important role in the age estimation of an individual. Dental age estimates are considered more reliable than bone age estimates as dental tissues show less vulnerability to taphonomic destructions and remodeling events. Different morphological stages of dental mineralization status correlate with the different developmental phases of life. Various age estimation methods such as Demirjian, Nolla, Willems, Cameriere, Kvaal, and others have been proposed in dental anthropology to be used with appropriate modifications for individuals of different age groups in different populations. However, each such method has its own advantages and disadvantages to be considered differently in bioarchaeological and forensic age estimations. This presentation will highlight various aspects of morphological, biochemical, and radiological methods in forensic dental age estimations, as will their scopes, accuracy levels, and limitations.

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**Forensic Anthropology/Odontology, Various Age Estimation Methods, Advantages and Limitations**

### G39 Disaster Victims Identification (DVI) Using Digital Radiology: A Case Report of a Brumadinho Victim Identification by the Dental Comparative Method

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**Learning Overview:** The goal of this presentation is to demonstrate, through a literature review and case report, the importance of the presence of a digital X-ray device for the comparative dental study within the Legal Medical Institutes and how the method is relevant in DVI, plus the benefit of using this instead of other identification methods.

**Impact on the Forensic Science Community:** The scrapping of Legal Medical Institutes is a known reality in Brazil. Lack of equipment and the devaluation of coroners is a barrier to the successful identification of dead bodies. Bringing this problem to the center of the discussion can be decisive in revising the amount of investment in this sector. In this context, this presentation will impact the forensic science community by revealing the importance of an X-ray machine in the work of the dental professional and how it can directly impact the rapid and accurate identification of bodies in mass accidents.

A mass disaster is a sudden natural or man-made event that sets in motion multiple work teams. The identification of corpses in these disasters is a process that involves, in addition to technical and scientific procedures, affective issues related to the families involved, legal procedures, and other activities.<sup>1</sup>

Dental structures are highly resistant to destruction, but dental identification also depends on relying on all available Antemortem (AM) dental documentation of the missing person. The role played by dentists in Postmortem (PM) dental examinations is extremely objective, and the use of dental radiology is an absolute need. AM radiographs can be easily compared with PM radiographic images, because even in the absence of dentistry or prosthesis work, the images offer very personal anatomical details.<sup>2</sup> The advancement of microelectronics and informatics, allied to the reduction of the cost of equipment, allowed the development of new, more powerful, and reliable techniques for comparing radiological images with forensic dentistry application.<sup>3</sup> Radiological scanning with digital X-ray before the necroscopic examination is useful for a quick overview of the remains and provides an easy method to record the data found in their *in situ* state.<sup>4</sup>

Dentists from the Forensic Anthropology Sector of the Legal Medical Institute of Belo Horizonte (LMI-BH) were assigned to perform a dental anthropological examination of an unknown corpse from the region where the Feijão Mine dam was ruptured, located in the city of Brumadinho in Minas Gerais, on January 25, 2019. After due expert procedures, dental data were collected and recorded in an expert report. Digital radiographs of the mandibular remnant were taken and archived at the LMI Forensic Radiology Service. Based on the comparative study of the occurrences and dental work present in the radiographic images of the mandibular remnant with the AM radiographic images, it was found that there was compatibility between AM and PM data. The characteristics revealed were sufficient, in isolation, for the effective recognition of the screen. Thus, it was concluded there is express need for the existence of digital X-ray devices for forensic purposes within the Legal Medical Institutes. Human identification by dental techniques is effective, more agile, and less expensive; however, in cases of cadavers without AM dental parameters for comparison, it is not possible.<sup>5</sup>

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#### Disaster Victims Identification, Dental Identification, Forensic Radiology

### G40 A Data-Driven Process, Prediction, and Reporting Model to Improve Human Identification Using the Mobile Application Intelligent System in Automation of Legitimated Examination Methods (iSALEM)

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**Learning Overview:** After attending this presentation, attendees will be informed about the development of the new innovative mobile application iSALEM. This will assist in the use of legitimate age estimation methods in a systematic form using digital automation as integrative new conceptual models to estimate and identify the age of the individual.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by acknowledging the challenge of clarity in the standard procedure to acquire estimated age. The iSALEM application is the response to usability and also the adequacy of using both the sciences and expertise to deliver a quality evaluation record.

The innovative application is part of the plan presented at the International Organization for Forensic Odonto-Stomatology (IOFOS) Conference titled “A Roadmap Plan of Developing Forensic Odontology in UAE” in 2017. Presently, the conversion of medicolegal data into a useful tool is essential to improve the process of analysis and outcome prediction by building a unique structural platform. The platform conveys tested models, techniques with specific formulas, tables of statistical values, and diagrams and X-ray films to create an impactful innovated application. iSALEM has two developing phases. In the first phase, an algorithm is used to automate the data from international practice of age estimation methods. In the second phase, a specific algorithm is intended to detect specific tooth development in medical images called image-recognition. Machine learning allows decision making to detect specific tooth development. Experts will be able to have access to a deep-learning Artificial Intelligence (AI) system for detecting developmental tooth stages.

Dealing with the many details of morphological tooth characteristics and numerous age estimation methods, iSALEM simplified the process to predict the age. The iSALEM application contains some of the most validated methods with technical procedures to narrow the search parameters and thereby assist in human identification of the deceased and living persons. Minimizing invasive assessment is essential to preserve the evidence and to reproduce the test. Therefore, radiological imaging in this process is a vital tool for iSALEM application. The new design is not only reducing the variation of error, but also enhancing accurate reporting. This paperless operation can protect case integrity of misfiling and prevents loss of immediate information by enhancing the use in the clinic, morgue, laboratory, and the area of disasters for DVI purposes. The new model’s results in the application were verified and validated for the intra-examiner by comparing the results of the age estimation model from iSALEM mobile application against the classic age estimation from experts in the field.

Authors of peer review publications set out age estimation methods for specific age phases and defined minimum requirements to perform an evaluation and reference to data and studies. Examiners should handle these necessities by allocating a case for a specific examination technique and interpreting its results. It is crucial to explain the method in the submitted report and its significance to the competent authorities. The model established in iSALEM using a mobile application is verified by the referees of the original paper, which makes it unique and the only data analyst program of its kind. It helps in reducing the subjective assessment of tooth development of the conducted subject and a comparison of the following criteria.

Dental age estimation narrows the scope in identifying unknown persons, which plays a significant role in many aspects of humanity, medicine, security, and sport. Using the iSALEM application, which is accessible software any time or place using smart phones, allows the consensus of experts internationally for improvements in methods and approaches. Furthermore, the great advantage of the process in the application is helping to calibrate examiners through exercise on solved cases before conducting a real case, which increases the examiners’ competences. Because of each methodology’s disadvantages as well as the advantages, it is essential to reduce the variation of errors in using these methods with communication technologies. The iSALEM application follows an already verified technique to produce a reliable and accurate dental age estimation report.

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#### **iSALEM, Age Estimation, Artificial Intelligence**

## G41 The Estimation of Sex From Dental Arch Dimensions: An Odontometric Analysis

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**Learning Overview:** The goal of this presentation is to educate attendees concerning the applicability of dental arch dimensions in sex estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing attendees to estimate sex from dental arch dimensions in the absence or extreme decimation of more reliable parameters or in association with other criteria.

Identification process is the backbone of crime investigation. Lack of sophisticated facilities such as DNA profiling in the developing countries make anthropometric and odontometric techniques a popular and useful choice for identification among the experts.

Estimation of sex is a part of the identification process and is an essential investigation in medicolegal examinations, especially those involving dismembered and skeletal remains. Principle determination of sex is based on well-established size differences between males and females. These differences are dependent on a variety of factors, such as genetics, environmental influences, etc. Traditional methods of determination of sex include observation of differences in morphological features of males and females. Most of these differences are somewhat subjective and are not measurable. However, owing to the reliable results derived in the research involving metric analysis, experts rely more on the methods which involve measurements.

Teeth are one of the most durable and well-protected parts of the human body that resist decay and destruction even in cases of extensive fire and can be used to determine the sex of an individual. Though a lot of research has been conducted on correlation of sex and tooth size, not much emphasis has been given to the use of dental arch dimensions for estimating sex. Even among the few studies that have conducted some research using dental arch dimensions, focus has been singularly on the inter-canine distance and not the distances between the premolars and molars, which are more likely to be found intact in decomposed and skeletonized remains. Also, the majority of such studies have been conducted only on the mandible. Measurements of maxillary arches assume importance, considering that the mandible may not always be recovered in extreme decay and dismemberment, and when only skull is brought for examination.

This present study was conducted to analyze the differences between males and females based on all the dental arch dimensions of both of the jaws in an Indian population. The dental arch dimensions of the canines, premolars, and the molars of both the jaws were recorded in dental casts of 307 participants. Comparison of the dental arch dimensions of males and females was performed and logistic regression models were generated to estimate sex. Receiver Operator Curve (ROC) analysis was performed to find out the potential to estimate sex for all the dental arch dimensions in both sexes. It was observed that maxillary inter-molar II distance gave the best results in estimating the sex. The sexing accuracy is seen to increase as one goes distally from canines to molars in each jaw; minimum for inter-canine distance and maximum for distance between the 2<sup>nd</sup> molars. The development of models for estimation of sex from dental arch dimensions will enable sexing in cases of dismembered skeletal remains too.

### Dental Arch Dimensions, Sex Estimation, Forensic Odontometry





## G42 Cementum Annuli: Technique, Microscopy, and Assessment of Age

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**Learning Overview:** After attending this presentation, attendees will understand: (1) the method of dental cementum thin section preparation of forensic teeth; (2) the microscopic analysis necessary to assess age via band qualification and quantification; and (3) age assessment methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing understanding of the techniques and methods. Attendees will be able to initiate the cementum annuli age assessment technique using microscopy in cases of partial representation of the dentition and skeleton.

Mineralized tissue, particularly the dentition, possess superb postmortem longevity. In certain wildlife such as bears and wolves, cementum annuli counts have been considered one of the most accurate indicators of age. While cementum annuli studies of zooarchaeological and modern wildlife are well documented, application to human teeth has been histologically and methodologically far less successful, preventing standardization and utilization. However, recent research indicates that cementum annuli may be used more reliably than any other part of skeletal remains to accurately determine age.

This study documents the laboratory preparation and enumeration of cementum structure on all tooth types and root locations using dissecting, light, and scanning electron microscopy. Teeth that were extracted because of periodontal disease, orthodontic, and prosthetic reasons were used in this study. The extractions ( $n=108$ ) from several general dental and oral and maxillofacial practices were documented with age, sex, and ancestry. All deciduous and permanent tooth types were embedded, thin sectioned (whole tooth, labiolingual and root, transverse, and mesio-distal), ground, polished, and etched or stained. Select intra- and inter-root locations (i.e., cervical, mid-root, apical, and inter-radicular) were examined. At low magnification, the dissecting microscope at 5X–10X was utilized to assess cementum quality, thickness, and root coverage. At high magnification, the scanning electron microscope at 100X–200X documented the structural reality of annuli and not for the counting procedure for age estimation. The light microscope at 25X–50X provides the tool for annuli counting in assessment of age.

Light microscopy of the mid-root area, regardless of the molar's mesial or distal root or tooth type, revealed the most easily visualized annuli for quantification. The cervical region is many times damaged or thinned from periodontal events, and the thickened apical region display a more "confusing" and unassignable array of annuli.

Countable cementum annuli are present in human teeth. Images of the annuli in the form of dark and transparent bands were counted using image analysis software to arrive at an age estimation. The average number of years of eruption for each tooth were added to the annuli count. Results helped determine the best methods to count the cementum annuli while also showing a significant correlation between the annuli count, predicted age, and actual chronologic age of each individual. Further studies are needed to help determine the environmental effects such as climate, temperature, and length of day on the production of the annuli in different populations.

### Cementum, Age, Microscopy

## G43 Morphological Examinations of the Teeth and Jaw Fragments Retrieved From an Ajnala Skeletal Assemblage: A Forensic Odontological Study

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**Learning Overview:** After attending this presentation, attendees will be sensitized regarding the postmortem longevity of dental remains and the significant contributions teeth and jaw fragments make toward identification of badly damaged and commingled human remains found in forensic situations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees how teeth serve as crucial markers of identity of the victims whose badly challenged human remains were excavated by amateur archaeologists or curiosity-seekers.

Bones and teeth have been increasingly used to figure out the biological profile of an individual(s) who lived in the past as they have the longest postmortem longevity to serve as crucial markers of human form and identity after death. Fragmented and commingled skeletal remains retrieved during mass disasters, archaeological excavations, construction activities, criminal submissions, forensic contexts, etc., pose a serious challenge for their identification endeavors in forensic anthropology. Odontology has contributed significantly in identification strategies of such unknown human remains as teeth remain minimally affected by biological, physical, or taphonomic destructions such as fire, chemicals, trauma, burial environments, etc.<sup>1</sup> Teeth contain invaluable biological, physical, and chemical information, crucially needed for responding to various forensic anthropological parameters such as provenance, biological identity establishment, exposure to pollutants or taphonomic/traumatic insults, estimation of dietary practices and subsistence patterns, paleopathology, etc. It is the small size and mineralized status of human teeth that impart their resistance against most types of decompositions and degradations.

Mankind has witnessed several heinous crimes against humanity, and some pre-existing structures such as abandoned wells, waterways, sewage systems, potholes, caves, pits, natural ravines, and roadside trenches have been commonly used as preferred burial sites for the clandestine disposal of human cadavers to avoid the attention of human rights watch-groups. In one such incident, thousands of badly damaged and commingled human remains, along with numerous contextual items of personal identity, were excavated non-scientifically by amateur archaeologists from a disused well found underneath a religious structure at Ajnala, India, in April, 2014.<sup>2-3</sup> The written records mention that the exhumed human remains belonged to 282 Indian-origin soldiers killed in August 1857 whose corpses were immediately dumped into a bricked well due to the contemporary socio-political situation in the area. Only teeth and a few jaw fragments could survive the taphonomic destructions during the hurriedly carried-out excavation of the site by the local curiosity seekers.

In this presentation, 1,527 teeth (1,200 dislodged and 327 present in 93 jaw fragments) collected from Ajnala skeletal assemblage were assessed for their age at death, sex characteristics, population attributes, traumatic and pathological signatures, occupation markers, health conditions, and other attributes. The application of three methods of adult age-at-death estimations (Johanson attrition grades, Average Stage Attrition (ASA), and Pulp-to-Tooth area Ratio (PTR)) found that the Ajnala remains belonged to individuals who were most likely from 32 years to 53 years of age (approximately). Johanson and PTR methods provided higher and lower age estimates, respectively, whereas the ASA method gave age estimates in-between the two other methods.<sup>4,5</sup> The anatomical discrete dental traits of jaw fragments revealed that at least 84% of them belonged to males, suggesting the vast majority of remains exhibit very prominent and obvious male characteristics. The odontometric values of dislodged teeth were substituted in the available logistic regression formulas generated from tooth metrics of known biological credentials, and it was found that about 78% of tooth measurements were categorized as males. Eleven non-metric dental traits, as defined by Scott and Turner, were evaluated following Arizona State University (ASU) Dental Anthropology System to estimate their population affinity.<sup>6</sup> It reaffirmed that predominance of relatively unique non-metric dental traits in Ajnala teeth allude them to be of Indians.<sup>7</sup> Only 4.2% of the dislodged teeth and 14% of the jaw fragments exhibited dental caries, which is in stark contrast to the 78% caries in present-day Indian populations; 4.9% of the teeth exhibited fracture of the crown or root, probably owing to their prolonged burial and possible ambient pressure exerted by soil and its components. The tooth wear in anterior teeth and premolars of jaw fragments may be attributed to some influence of biting of cartridges for probable loading into rifles/muskets (perhaps). Extrinsic blackish-brown or reddish staining on a few teeth were suggestive of nicotine consumption, smoking of any form, or betel-nut chewing/eating habits.<sup>8</sup> These observations correspond to the biological and geographic identity of the Ajnala skeletal remains as described in the written records. The detailed results will be presented in the textual, graphical, tabular, and diagrammatic forms.

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### Forensic Odontology, Ajnala Skeletal Remains, Age and Sex Estimations

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### G44 The Importance of Bitemarks in Child Abuse Cases in Turkey

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**Learning Overview:** After attending this presentation, attendees will have learned about the challenges in collecting and reporting child abuse cases in Turkey.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing some of the injury types in child abuse cases.

Child physical abuse is a major cause of pediatric morbidity and mortality that is associated with physical and mental health problems and can last until adulthood. There are limited numbers of reported child abuse cases in Turkey. Some of the reasons for this lack of reporting child abuse cases include a lack of adequate training of health workers, hesitance to interfere with family issues, the belief that a parent cannot hurt their own children, a lack of official centers for reporting child abuse, and inadequate record keeping and reporting of these types of abuse cases. Turkey is a country that bridges Asia and Europe, acts as a passageway for immigrants, and is also a place for their settlement. Due to the increase of immigrants in recent years, the importance of reporting and the correct handling of child abuse cases has taken more prominent place in the country's justice system.

Child abuse case injuries include facial injuries caused by slapping, patterned injuries, lip lacerations, scaring of the corners of the mouth due to gag use, bruises, human bitemarks, electrical, chemical, or thermal burns, maxillary or mandibular fractures, intraoral injuries, mobilized or broken teeth, torn frenilumlar, other intraoral lacerations, and bruising and injuries to the hard and soft oral palate from forced oral sex.

**Case 1:** A 3-year-old boy—a forensic report prepared by an emergency room doctor was sent to legal authorities. Upon further examination of the boy, an official report of a child abuse complaint was filed. Examination revealed fracture of the right tibia proximal, bruises on the left lower quadrant of the left breast and right kidney area, a circular pattern of bitemarks on the left gluteal area, scars measuring 2cm x 1cm on the left index finger, swelling of the right arm, and swelling and bruises on the left leg. The report included a list of 25 different injuries. It also concluded that the scars and bruises resulted from repeated blunt trauma and human bites. A forensic bitemark analysis performed by the Istanbul Forensic Medicine's Dental Department was included in the report and used in identifying the defendant.

**Case 2:** A 2-year-old boy—death resulted from child abuse. According to toxicological analysis, there was no evidence of any chemical or substances that were sought in the analysis. There were numerous nail marks and bruises on the neck, and evidence of continual physical abuse were present on the trunk and victim's back. The cause of death was blunt head trauma that caused cerebral hemorrhage and brain tissue damage. The victim also had rib fracture, internal organ, and abdominal injuries. Upon examination of the bitemarks, the child's father was a match for this closed case.

#### **Bitemarks, Child Abuse, Turkey**



## G45 A 3D Analysis of Bitemarks: A Validation Study Using an Intraoral Scanner

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**Learning Overview:** After attending this presentation, attendees will have learned a new 3D approach to human bitemark analysis using an intraoral scanner. With this approach, the feasibility, reproducibility, and reliability of comparisons were established.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how a 3D analysis optimizes and facilitates bitemark analysis.

**Purpose:** Conservation of the 3D aspect of bitemarks improves their analysis. In clinical dentistry, intraoral scanners are used as an alternative to conventional dental impressions for prosthetic design. Digital impressions are accurate, editable, and stable. In forensic odontology, an intraoral scanner could be used to record the bitemarks and a suspect's dentition. Then a 3D point processing software could compare these two objects.

The goal of this presentation is to assess the feasibility, the reproducibility, and the reliability of this new approach to bitemark analysis.

**Materials:** Twenty-seven volunteers bit into four materials: a triple thickness of dental wax, a hard cheese (Gouda or similar), a bar of milk chocolate, and an apple. The intraoral digital scanner PlanMeca® Emerald scanned dentitions and bitemarks. The scans obtained were exported into the mesh comparison software CloudCompare®, which can generate overlays automatically. For this purpose, dentitions were aligned and compared with the indentations. Meshing and non-meshing were visualized using a colorimetric scale.

**Methods:** First, the same blinded observer analyzed 256 comparisons for each material in order to compare the bitten supports. Second, 50 comparisons of bitemarks from wax were analyzed in a randomized blinded study involving four observers (dentist, dental student, forensic scientist, and police officer). None of the observers were trained before making these comparisons. Descriptive statistics, such as predictive value analysis and Cohen's kappa coefficient, were carried out in order to study the reproducibility of comparisons.

**Results:** For all materials, the rate of correct classifications was higher than 90%, and misclassification was rare. After calculation of predictive values, the wax support obtained the best result (positive predictive value: 87.1%, negative predictive value: 100%) and the apple the worst (positive predictive value: 57.1%, negative value predictive: 98.6%). Moreover, for each observer, negative predictive value was near 100%. Positive predictive values were more variable but higher than 70%. Cohen's Kappa coefficients showed a better concordance (almost perfect agreement) between the two observers whose training included odontology. For the other observers, the coefficient was substantial or agreement was perfect. Positive predictive values were better for the dentist and dental student than for the forensic scientist and police officer. Thus, the work clearly highlighted the importance of experience in odontology.

**Conclusion:** The 3D aspect of bitemarks was preserved, which facilitated analysis and reduced bias. Thus, 3D analysis was more objective than a photographic method. Dentitions not implicated by bitemarks could be excluded with certainty. Moreover, dental experience was fundamental to obtaining the best values.

### **Bitemarks, 3D Analysis, Predictive Value**



## G46 3D Dental Digital Study Models in Bitemark Comparison

Robert B.J. Dorion, DDS\*, Laboratoire S.J.M.L., Montreal, PQ H2K 3S7, CANADA

**Learning Overview:** After attending this presentation, attendees will have acquired information regarding the role that 3D dental digital study models have in bitemark comparisons.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that 3D dental digital study models are as accurate as original dental stone casts and have the distinct advantage of being unbreakable, cost-effective substitutes for the original dental stone casts and can easily be securely transferred to forensic dental experts and other interested parties by the internet.

A male adult defendant was sent to trial on charges of first-degree murder, aggravated sexual assault, and sexual interference in connection with the death of a three-year-old female. There were 13 human bitemarks on the torso and limbs, in addition to three scarred/healing human bitemarks to the buttocks and right forearm.

The case went to preliminary inquiry in 2010 and to trial in 2012. A Board-certified forensic dentist testified for the prosecution in both events. The trial court judge acquitted the accused, and the Court of Appeal ordered a new trial on the basis of judicial error. There was no dental expert witness for the defense, neither at the preliminary inquiry nor at trial. For both the preliminary inquiry and for the trial, the case was considered a “closed population” event. However, for the retrial, further investigation discovered that more persons had been in contact with the child than had originally been thought, thus it became an “open population” case. A forensic dental expert witness for the defense was appointed for the scheduled retrial in 2014.

For the retrial, a judge ordered the duplication of original dental stone casts of six suspect dentitions to be constructed in another jurisdiction and ordered that the duplicate dental stone casts be sent to all experts in the case. The pairs of duplicate dental stone casts received by the forensic dental expert witness for the defense were defective in one form or another. One dental stone cast contained three broken anterior teeth on the stone models, which the suspect dentition did not have. Others had air bubbles on the incisal edges of anterior teeth.<sup>1</sup> In the absence of the original dental stone models, it was not possible to determine if the defects were artifact on the original or the duplicate models. Either way, air bubbles do not exist on human teeth, and the models cannot be used for comparison.

The use of digital files representing 3D objects is currently accepted for diagnostic and comparison purposes and are recognized for judicial purposes. Had 3D dental digital study models been made from defect-free dental stone casts and transferred to the interested parties, this dilemma would not have occurred.

This presentation will include the use of different views of 3D dental digital study models, 3D measurement tools, cross section properties, and cast “highlights” of the ORTHOpix™ 3D dental digital study models for comparison purposes.

In conclusion, 3D dental digital study models can play an important role in bitemark comparison and can substitute for the original dental stone casts of suspect dentitions.

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- <sup>1.</sup> Robert Dorion. The Anatomy of an Aborted Retrial Involving Bitemark Evidence. *Proceedings of the American Academy of Forensic Sciences*, 68<sup>th</sup> Annual Scientific Meeting, Las Vegas, NV. 2016.

**Forensic Odontology, 3D Digital Dental Study Models, Bitemark Comparison**



## G47 Non-Bitemarks That I Have Known

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**Learning Overview:** The goal of this presentation is to focus on the first step of a three-step process outlined by the American Board of Forensic Odontology (ABFO) with respect to new bitemark guidelines and a bitemark decision tree/algorithm, the first step being whether a patterned injury is in fact a bitemark. Several cases will be presented in which a patterned injury looked to someone to be a bitemark that later was determined to not be a bitemark and the motivation that led some investigators to believe that a pattern injury was a bitemark.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by postulating that if the decision tree proposed by the ABFO is used appropriately, far fewer cases will advance beyond the first step of the suggested guidelines, ultimately preventing costly judicial errors.

The long and winding road of bitemarks began with *Doyle v. the State of Texas* in the 1950s—a bite in cheese<sup>1</sup>. Over the ensuing years, the ability to “match” the teeth of an individual, not to cheese but to a human dermal bitemark was, if not the stated goal, the unstated goal of both bitemark analysis and comparison. It was also considered, as many forensic disciplines were, both straightforward and scientifically valid. It was not scientifically validated—and many think scientifically invalidated. In the years since *Doyle v. the State of Texas*, there have been numerous cases in which bitemark analysis and comparison resulted in convictions of persons that were later overturned. In excess of 30 people, wrongfully convicted of crimes in which bitemark evidence was used, have been re-adjudicated. Finally, bitemark evaluation, analysis, and comparison is only a small part of many forensic odontologists’ case work, with the largest part of most forensic odontologist practices being comprised of the identification of human remains.

In 2018, the American Board of Forensic Odontology published new bitemark guidelines and a bitemark decision tree/algorithm. The goal of this endeavor was to guide the profession with respect to the three-step process by which an investigator first decides whether a patterned injury is a bitemark, second whether a mark that is deemed to be a bitemark possesses evidentiary value sufficient to warrant further investigation, and third, having passed the previous two hurdles, whether a suspect dentition or dentitions can be excluded or not excluded as having produced the bitemark.

This presentation intends to focus only on the first step, since often the question before an investigator or even before the courts is not always “whose bitemark is it?” but “is it a human bitemark at all?” Several cases will be presented in which the first fork in the road was reached (i.e., a patterned injury looked to someone to be a bitemark that later was determined to not be a bitemark). It is postulated that if the decision tree proposed by the ABFO is used appropriately, that far fewer cases will pass the bar of even being a bitemark and certainly for evaluation for evidentiary value sufficient to warrant further investigation; and even fewer will reach the stage of comparison to a suspect. This will be accomplished by examining non-bitemarks that were thought to be bitemarks, the motivation that lead some investigators to believe that a pattern injury is a bitemark, and an assessment of some of the clinical cases in which a pattern of contusions, lacerations, incisions, or abrasions led to someone at the very early stage of an investigation to diagnose a patterned injury as a bitemark.

With the human cost of a wrongful conviction being terrible enough, even from a monetary standpoint of the cost of a jury trial, the post-conviction appellate costs, and monetary awards to those wrongfully convicted, there is a strong argument for applying the 2018 algorithm effectively since it seems prudent to look at a decision-point where at least some of these cases can be filtered from the judicial system.

### Reference(s):

<sup>1</sup>. *Doyle v. the State of Texas*. 329 S.W.2d 286 (1959).

### Bitemark, ABFO, Wrongful Conviction



## G48 A Historic Perspective of Bitemark Analysis and Bitemark Comparison

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**Learning Overview:** After attending this presentation, attendees will possess a historic perspective of bitemark analysis and bitemark comparison.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the myriad of changes that have occurred from the 1970s to today. These include, but are not limited to, the American Board of Forensic Odontology (ABFO) establishing a Research Committee, the Standards, Methods, and Procedures Committee, the Bitemark Evidence and Patterned Injury Committee, the Certification and Examination Committee, and Bitemark Workshops.<sup>1</sup> As well, the American Society of Forensic Odontology (ASFO), the American Academy of Forensic Sciences (AAFS) Odontology Section, and the ABFO are all involved in continuing education for their members.

The influence of 2009 National Academy of Sciences (NAS) recommendations, the National Institute of Justice (NIJ), the President's Council of Advisors on Science and Technology (PCAST) conclusions, the Texas Forensic Science Commission report, and finally the National Institute of Standards and Technology (NIST) Organization of Scientific Area Committees (OSAC) efforts to open dialogue and promote consensus are discussed.<sup>2-5</sup>

The role that universities worldwide have contributed, the textbooks and scientific articles published, and the research in the field since the 1970s is ever expansive and contributes to the advancement of bitemark evidence.

For some, bitemark analysis was synonymous with bitemark comparison in the 1970s, and wrongful convictions resulted. For others, the exaggeration of claims of certainty of identification were not justified. Fortunately to date, there have been no reported, nor cited, wrongful convictions involving bitemark evidence anywhere else in the world other than in the United States. This suggests that a multitude of domestic systemic problems contributed to wrongful convictions of bitemark evidence in the past.<sup>6</sup>

If class and individual characteristics exist, and both sufficient quality and quantity of information is present (evidentiary value), a diagnosis can be made that the pattern was created by teeth or was not. On the other hand, there may be insufficient information to arrive at a diagnosis. Bitemark comparison may or may not follow, depending on circumstance.

In conclusion, bitemark analysis and bitemark comparison are two different topics requiring individual assessment. With the advent of DNA, and the new algorithms for bitemark analysis and for bitemark comparison, a completely different paradigm exists from that of the 1970s.

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5. *The Texas Forensic Science Commission. Forensic Bitemark Comparison Complaint Filed by National Innocence Project on Behalf of Steven Mark Chaney—Final Report*. April 12, 2016.
6. Epidermis and Enamel: Insights into Gnawing Criticisms of Human Bitemark Evidence. *Am J Forensic Med Pathol*, 20018 39:2:87.

### Forensic Odontology, Bitemark Analysis, Bitemark Comparison



### H1 Circumstances and Injuries in Female Homicide Victims of Strangulation: A Four-Decade Review of 143 Cases in King County, Washington

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**Learning Overview:** After attending this presentation, attendees will be able to: (1) describe the circumstances surrounding homicides of female victims of strangulation, (2) list the most reliable physical evidence supporting a diagnosis of strangulation, and (3) explain why females are especially vulnerable to this modality of violent death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing data from a large case series of female homicide victims that can help in identifying circumstances and physical evidence characterizing this modality of death of which females are especially vulnerable.

Strangulation is a cause of death that is often challenging to establish because of relatively minor anatomic injuries, obscuring postmortem changes, and coexisting injuries, natural disease, or intoxication. Previous studies have identified characteristic findings in both decedents and surviving victims, demonstrating considerable variation in the anatomic findings, as well as the difficulties in proving death or injury in strangulation. Thus, it is important to review a large series of cases to more completely understand the complexities inherent in making a diagnosis of strangulation.

This study is a retrospective review of homicidal strangulation of female homicide victims examined in the King County Medical Examiner's Office (KCMEO) from January 1979 to December 2016. For the cases identified, demographic information was collected from the medical examiner files, investigative reports were used to assemble circumstantial information, and autopsy reports were abstracted to compile anatomic injuries. Data from the Washington Attorney General's Office Homicide Investigation Tracking System supplemented the medical examiner information regarding perpetrator and circumstances. The current KCMEO electronic database was queried to compare the number of female strangulation homicide victims relative to those of males from 2000 to July 2019.

Over the time period of 1979 to 2016, there were 143 female homicide victims of strangulation used for this study. In the later time period used to compare the number of male to female strangulation victims, there were 30 females and 9 males. In the 143 cases of female victims, the highest proportion of cases (29%) were in the age range of 20–29 years; 14% of cases were in ages older than 70 years. Sexual motivation was prevalent in the younger age group, whereas robbery prevailed in the older age group. The majority of deaths were in private places (65%) and the perpetrator was known to the victim in 80% of cases. Of the known circumstances surrounding the homicide, 25% were related to domestic violence; sexual assault was evident in at least 25%. Overall, manual strangulation was the predominant method of strangulation; however, in cases with sexual motivation, ligature strangulation predominated. In approximately 73% of cases, strangulation alone was responsible for death; other modalities of injuries were present in the remainder, most commonly blunt force injury.

Petechiae were documented in more than 80% of the cases, nearly always in the conjunctivae; postmortem decomposition likely obscured their appearance in other cases. Internal neck injuries were evident in about 92% of the cases, mostly in the form of soft tissue and strap muscle hemorrhages (86%); thyroid cartilage fractures were noted in 38% of the cases, and hyoid fractures occurred in 25%. The latter especially were highly correlated with ages older than 40 years.

The results of this study are largely consistent with previous studies, showing that female victims of strangulation outnumber those of males by a ratio of at least 3:1. Circumstances and venues of domestic violence account for a substantial proportion of female homicidal strangulations, and sexual motivation is also a major factor, readily explaining why females are especially vulnerable to this modality of death. Conjunctival petechiae and soft tissue/muscle neck injuries remain the best evidence supporting a diagnosis of strangulation, while cartilaginous or hyoid fractures are much less common, especially in younger victims.

#### Strangulation, Female, Injury Patterns





### H2 A Fistful of Dollars: An Unexpected Delayed Development and Rupture of a Traumatic Fusiform Posterior Inferior Cerebellar Artery (PICA) Aneurysm

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**Learning Overview:** After attending this presentation, attendees will understand the importance of suspecting and evaluating vascular lesions of the posterior cranial fossa following closed head trauma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that a craniocervical trauma that creates acceleration and deceleration of the cerebellum inside the skull affecting PICA at its origin can lead to the formation of intracranial traumatic aneurysms, causing ischemic processes and death of the affected subject.

Intracranial traumatic aneurysms are extremely rare, representing less than 1% of all intracranial aneurysms. Traumatic aneurysms should be suspected in the context of acute neurological deterioration following any type of closed head injury. Most of these lesions develop in the anterior circulation (i.e., in the middle cerebral artery, in the anterior cerebral artery, or in the internal carotid artery). Less than 10% of them occur in the posterior circulation. Delayed rupture occurs in about 50% of cases; the interval between injury and rupture ranges from a few hours to ten years, with an average interval of 14–21 days. The mortality rate is high, ranging between 32%–54%. As far as pathogenesis is concerned, it is suspected that the head trauma produces acceleration and deceleration of the cerebellum within the skull, tearing the PICA at its origin.

A 26-year-old man was brutally punched in the face in a disco. He was admitted to the emergency department unconscious, with a lacerated-contused eyebrow wound, and with several vital sign parameters that were severely altered. No skull fractures were evident. A head Computed Tomography (CT) scan revealed extensive subarachnoid hemorrhage, cerebral edema, intraventricular hemorrhage, and post-traumatic endocranial hypertension. Based on these diagnostic findings, the patient underwent bilateral decompressive neurosurgery. There were no complications until postoperative day ten, when suddenly the patient's hemodynamic status and neurologic condition began to worsen. Head CT scan and CT angiogram were performed showing a fusiform aneurysm measuring approximately 3.6 x 4.5mm located in the proximal portion of the left PICA. This aneurysmal dilatation showed homogeneous enhancement in the arterial phase and presented irregular profiles on the anterolateral side due to apparent rupture. Associated hypodensity of the brainstem due to vasospasm phenomena was identified, as was a vasospasm-associated filiform aspect to the arteries of the Circle of Willis. Overall, the described findings were compatible with a ruptured post-traumatic intracranial aneurysm, a rare but recognized complication of closed head trauma. Despite neurosurgical attempts to repair the vessel, the patient died after a few months following multiple episodes of massive rebleeding.

Diagnostic features useful to distinguish a traumatic aneurysm from a natural fusiform aneurysm include age and sex (i.e., traumatic aneurysms are more frequent in young males) and a history of closed head trauma with typical angiographic aneurysm characteristics, including peripheral position. Other supportive traumatic aneurysm features include aneurysm location away from a branching point with irregular contour of the aneurysmal sac, absence of an aneurysmal neck, and delayed filling and emptying with position adjacent to the edge of the sicle. The radiological images obtained in the case described above fully reflected these characteristics.

In conclusion, this case report demonstrates that a history of a closed traumatic brain injury causing intraventricular hemorrhage with massive subarachnoid bleeding in the posterior cranial fossa should raise concern for significant vascular lesions, such as a ruptured aneurysm or other rupture/dissection of the vertebro-basilar arterial system. Unfortunately, it is not possible to precisely predict the timing for development nor rupture of a post-traumatic intracranial aneurysm; the relatively few cases presented in the literature, anatomical variability of the subjects involved, and multiplicity of other involved factors preclude standardized timing. Currently, careful monitoring of patients at risk seems the only option to detect intracranial traumatic fusiform aneurysms, a rare but potentially lethal consequence of closed head traumatic injury.

#### TBI, Subarachnoid Hemorrhage, Traumatic PICA Aneurysm



### H3 An Accidental Death Caused by Blunt Force Injuries Resulting in Multiple Fractures and a Pulmonary Fat Embolism

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**Learning Overview:** After attending this presentation, attendees will be familiar with the pathophysiology and microscopic appearance of pulmonary fat embolism, a known cause of death following trauma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting a case of accidental death related to multiple fractures and pulmonary fat embolism caused by traumatic blunt force injuries in a man with a known clinical diagnosis of coronary artery disease and hypertension.

A 71-year-old Caucasian male, with additional history of hypertension, coronary artery disease, and obesity, was the driver of a vehicle that crossed the centerline and collided head-on with a semi-truck. His legs were trapped, requiring extrication. Diagnostic imaging revealed a left iliac crest fracture extending into the sacrum with bilateral superior and inferior pubic rami fractures, left tibial plateau fracture, and right olecranon fracture. Although he was initially alert upon arrival, he experienced cardiac arrest while on the operating table and was pronounced dead.

A complete medicolegal autopsy was performed, which revealed an obese (Body Mass Index [BMI]=38) adult male with numerous blunt force injuries to the head, neck, trunk, and extremities. Internal examination disclosed multiple fractures involving the pelvis, ribs, and sternum, a 200mL right hemothorax, and a 100mL left hemothorax, but no other significant injuries. Cardiomegaly (800 grams) with marked biventricular dilatation was noted along with other findings consistent with hypertensive and atherosclerotic cardiovascular disease. Microscopic examination of the lungs included extensive intravascular fat emboli, which were also visualized with Oil Red O on frozen section. The cause of death was ruled as multiple blunt force injuries with multiple fractures and pulmonary fat embolism, with underlying hypertensive and atherosclerotic cardiovascular disease and obesity considered contributing causes of death. The manner of death was accidental.

Although a less common cause of death than pulmonary thromboemboli, fat emboli are an exceedingly common pathologic process known to occur in traumatic femur and pelvic fractures.<sup>1</sup> Mechanistically, fat globules are released from the bone medullary space into the venous circulation, which return to the heart and subsequently the lungs. Although their occurrence in association with traumatic fractures is common, symptoms related to traumatic fat embolism are less common; acute right heart failure can occur in fulminant cases, resulting in death.<sup>2</sup> Fat emboli are present in the microcirculation, which must be distinguished from the clinical manifestations of the so-called fat embolism syndrome. This syndrome includes common systemic signs, such as respiratory distress, altered mental status, and petechial rash, but strict diagnostic criteria are not agreed upon.<sup>3</sup> Autopsy diagnosis of pulmonary fat emboli can be accomplished with histologic examination of tissue samples, which may allow for postmortem diagnosis of fat embolism syndrome.<sup>4</sup> In certain cases of death related to injury, fractures with associated extensive fat embolism may be the major (or only) serious manifestations of trauma. Fat embolism, with or without contribution from underlying natural disease, is sufficient to explain death in such cases.

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#### Fat Embolism, Blunt Force Trauma, Death



## H4 Carbon Dioxide Asphyxiation Due to Pulmonary Embolism: A Case Report

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**Learning Overview:** After attending this presentation, attendees will be familiar with the findings in confined space carbon dioxide asphyxiation and understand the importance of autopsy findings and death scene investigation in determining cause of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing an unusual case of confined space asphyxiation as the result of pulmonary embolism and the importance of collaboration between death scene investigators, police, and the forensic pathologist for an accurate evaluation of these fatalities.

A 38-year-old man working as a special effects technician using a commercial Carbon Dioxide (CO<sub>2</sub>) tank to create a fog effect within a theatre was operating the shut-off valve in a contained room under the stage when he was unable to be contacted via portable radio for 12–13 minutes. He was found unresponsive with seizure-like activity by two coworkers, who also became unresponsive. A third coworker was able to turn off the CO<sub>2</sub>, allowing the removal of the three victims from the room. Both coworkers were revived and transported to the hospital along with the primary patient, who remained unresponsive. Arterial blood gas performed en route to the hospital revealed a CO<sub>2</sub> level of greater than 100mm Hg with the level decreasing to 40mm Hg upon arrival at the hospital. Therapeutic hypothermia protocol was initiated due to continued unresponsiveness and seizure-like activity. Upon rewarming, Computed Tomography (CT) of the head revealed severe encephalopathy, diffuse cerebral edema, and downward herniation. Brain death was declared, care was withdrawn, and death was pronounced four days after the incident.

Autopsy performed the following day revealed a thromboembolus within the main trunk of the pulmonary artery and completely obstructing the right pulmonary artery. A healing hemorrhagic pulmonary infarct, as well as multiple deeper shower thromboemboli and dilated cardiomyopathy, were also present. The right lower extremity was swollen and a massive residual organizing thrombus adherent to the deep vein endothelium was found. Microscopic examination of the pulmonary thromboembolus demonstrated interlacing gray fibrinous bands indicative of a long-standing organizing blood clot consistent with at least a week-long process. The deep vein thrombus from the right leg was similar in appearance. Further investigation of the decedent's history revealed a recent lower extremity injury. Based on the autopsy findings, it was concluded that the collapse at work was due to an episode of pulmonary embolization by deep vein thrombi, which partially occluded the pulmonary artery tree. The sudden incapacitation and collapse prevented the decedent from operating the shut-off valve on the CO<sub>2</sub> tank. The unencumbered release of CO<sub>2</sub> from the tank led to an exclusion of oxygen, resulting in confined space asphyxia and, ultimately, death due to anoxic encephalopathy.

CO<sub>2</sub> is a colorless, odorless gas present in low percentages in normal room air (0.02%–0.04%). At low concentrations, it has little toxicological effect.<sup>1</sup> At higher concentrations, it leads to increased blood pressure, tachypnea, tachycardia, respiratory acidosis, and cardiac arrhythmias.<sup>1,2</sup> It acts as both an asphyxiant and toxicant, causing neurological changes, such as headaches, clouded awareness, and impaired consciousness.<sup>1,2</sup> Seizure, coma, and death occur at concentrations greater than 10%.<sup>1,3</sup> At levels above 20%, there is a high risk of fatality.<sup>4</sup> At such high levels, loss of consciousness can occur within seconds, which can lead to severe accidents also involving rescuers, as in this case.<sup>4</sup> Deaths due to CO<sub>2</sub> often involve storage tanks or wine cellars as CO<sub>2</sub> is a by-product of fermentation, and the enclosed spaces allow the accumulation of high concentrations.<sup>2</sup> CO<sub>2</sub> has a greater density than oxygen, causing it to accumulate near the ground and displace oxygen from the area.<sup>1</sup> Asphyxiation occurs in the confined space as a consequence of an oxygen-deficient atmosphere.<sup>1</sup> Toxicity may be expedited by the body's failure to eliminate CO<sub>2</sub> resulting from respiratory failure or obstruction, such as pulmonary embolism as in this case. Autopsy findings are non-specific and the rapid accumulation of CO<sub>2</sub> after death renders postmortem CO<sub>2</sub> levels of little diagnostic value.<sup>5</sup> Therefore, the diagnosis of CO<sub>2</sub> intoxication is based on the scene investigation and circumstances of the death with the exclusion of other possible causes.<sup>1</sup> This is an unusual but not to miss diagnosis where close communication among all parties is critical, especially those participants at the scene.

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### Asphyxia, Dioxide, Embolism



### H5 Autopsy Findings in Dog Attacks in Mississippi

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**Learning Overview:** After attending this presentation, attendees will understand the affects the human body can suffer after experiencing a severe dog attack.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a series of five different deaths in which the victims went through the extensive trauma of a dog attack and will provide an insight into multiple ways a dog attack can lead to a decisive cause of death.

The intent of this presentation is to better characterize the injuries victims may sustain in cases of severe dog attacks. A series of five cases in which victims sustained extensive traumatic dog attack injuries are described and help provide insight into the multiple ways a dog attack may factor into cause-of-death determination.

**Case 1:** A 70-year-old White female was found in her backyard by a neighbor after discussing with him that his dogs had gotten out of his fence. Autopsy examination revealed multiple cuts and claw marks noted on the anterior and posterior of her body, along with nearly a complete stripping of the skin from each arm. This case presented the challenge of determining whether the victim had died of natural causes in her backyard prior to the attack or whether she died as a direct result of trauma from the attack.

**Case 2:** A 75-year-old African American female was known to have had an agreement with many of her neighbors to collect cans. It is believed that she knocked on the door of a house whose owners often had cans for her, and when she found them not home, she wandered into their backyard. She was found in the yard with the owner's two dogs who had attacked her and dragged her body to the back of the yard against the fence. Autopsy examination revealed the majority of her facial tissue to be missing, including both ears. It was determined that the intense trauma to her neck was most likely the ultimate cause of death.

**Case 3:** A 3-year-old African American male was found at autopsy to have severe lacerations of his face and throat, as well as lacerations and gnawing injuries to both ears and jugular veins. Additionally, he had puncture wounds on the backs of his thighs as well as on the back of his head and neck. In this case, the attack was so powerful that he was found to have a dislocation of the C1/C2 vertebrae.

**Case 4:** A 45-year-old African American male was transported to the medical examiner's office with no known history other than that the victim was found lying deceased in the street. The decedent's body was covered in irregular lacerations and tears and had been castrated. There was evidence of multiple sharp force and blunt force injuries. Due to lack of further conclusive investigative findings, the death was ruled a homicide due to homicidal violence.

**Case 5:** A 50-year-old African American male stated before arriving at the hospital that a neighbor's dogs had attacked him and chased him down the street. At autopsy, there were numerous puncture wounds of the body, particularly on the left side and extremities. There was tearing and ripping of almost the entirety of the inner arms. Law enforcement investigation—in conjunction with the medical examiner's office findings—subsequently revealed that the victims in Cases 4 and 5 were killed by the same set of dogs. The dogs' owner was later charged with manslaughter with culpable negligence for both attacks.

#### Trauma, Dog, Laceration



### H6 Frangible Ammunition in Gunshot Wound Suicides in the Hampton Roads Region of Virginia: An Upward Trend?

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**Learning Overview:** After attending this presentation, attendees will be familiar with frangible ammunition effects on imaging and at autopsy of gunshot wound suicides and will elevate their index of clinical suspicion for an upward trend in its use.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of a possible trend in the use of frangible ammunition in gunshot wound suicides and by recognizing that frangible ammunition, although intended to cause less bystander damage, inflicts devastating injuries at contact range and may produce exit wounds.

Frangible ammunition has historically been used by law enforcement for training and for domestic and Special Weapons And Tactics (SWAT) team interventions, as it is more likely than conventional ammunition to wound only the intended target. This report reviews three recent gunshot wound suicides with frangible ammunition in the Hampton Roads area of Virginia (a catchment area covering a population approaching one million).

Within a span of three days, three unrelated persons committed suicide by gunshot wound to the head with frangible ammunition. A 25-year-old man with a family-reported history of psychiatric problems shot himself in the side of the head with a .40 caliber pistol, after first shooting and killing another victim with conventional ammunition in a homicide-suicide event. A 70-year-old male hunter, with a history of traumatic brain injury and anger management issues, shot himself in the side of the head with a .38 caliber revolver. In the third case, a 38-year-old woman with a significant psychiatric history shot herself in the forehead using a 9mm handgun loaded with 50-grain lightweight bullets carrying an increased gunpowder load. The first two decedents were proficient in knowledge of guns and ammunition (i.e., the younger man from military service and the older man from hunting experience). The third decedent used ammunition found at the scene.

On radiographic imaging of each decedent, there were radiating fractures from the entrance wound and frangible fragments scattered throughout the cranial cavity; the intact base of the frangible bullet was always identified. Autopsy of the first two cases demonstrated characteristic pulpified gunshot wound tracks through the brain, accompanied by multiple smaller tracks from ammunition and disrupted bone fragments. Fragments were also found in the scalp and dura. The first case had no exit wound. The second case had a partial exit wound from escaping fragments. The third case, with a central forehead entrance, had a continuous entrance-exit wound of the entire frontal skull and scalp, with radiating fractures throughout the calvarium and base of the skull; this case had some characteristics of a high-velocity gunshot wound, due to the ammunition type.

**Discussion:** Gunshot wounds comprise the most common method of suicide in the United States for both men and women. Standard ammunition is generally used (fully or partially jacketed lead-core bullets). Such bullets may be stripped into fragments during passage through bone from shearing forces, or they may remain intact. In contrast, frangible ammunition is manufactured to break apart into smaller pieces upon impact with hard surfaces. This enables law enforcement to reduce the risk of any ricochet damage.<sup>1</sup> Frangible ammunition can be differentiated from sheared standard ammunition on X-ray films by the granular-appearing borders of the fragments, the intact base, and the absence of standard core and jacket fragments.<sup>2</sup>

Studies have found that the damage caused by frangible ammunition is similarly severe to the damage caused by standard ammunition; these three cases illustrate the extent of damage caused by frangible ammunition in hard-contact gunshot wound suicides, and demonstrate that fragments may exit.<sup>3</sup> These cases also illustrate that frangible ammunition can be recognized by its characteristic appearance on radiographs and at autopsy, and that there may be increasing use of frangible ammunition in gunshot wound suicides.

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#### Frangible Ammunition, Gunshot Wound Suicide, Forensic Autopsy



### H7 The Feasibility of an Enteroclysis Pump for Postmortem Computed Tomography Angiography (PMCTA)

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**Learning Overview:** After attending this presentation, attendees will be better informed regarding the use of an easy-to-use, inexpensive enteroclysis pump to acquire an accuracy of PMCTA in a clinical setting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a new PMCTA method for daily use in their own clinical practices.

PMCT is an established method utilized to diagnose disease, detect complications, and assist in determining cause of death in both clinical and forensic cases. By adding intravascular infusion of contrast medium, PMCTA can provide additional information regarding vascular structures and hemorrhage. If easy to operate and low in cost, this technique would likely be more frequently utilized and of additional value for clinical and educational purposes, particularly in the forensic scientific context. As in the Netherlands, all postmortem imaging is performed in a hospital setting, this study developed a new alternative technique using materials readily available in a clinical setting. As an alternative pump, this study chose an enteroclysis pump, as these are readily available and yet not in continuous use in clinical practice.

PMCTA was performed on ten decedents obtained via the anatomy department. First, a metal T-piece was inserted into the femoral artery as part of the standard practice for conservation. Second, surplus contrast medium with sodium chloride was infused into the body through a catheter tube set attached to the metal T-piece, using an enteroclysis pump obtained from the radiology department.

To assess PMCTA quality, the body was divided into nine regions as follows: head-circle of Willis (COW), carotid arteries, aortic arch, thoracic aorta, abdominal aorta, upper arms (brachial artery), lower arms (radial and ulnar arteries), upper legs (femoral artery), and lower legs (tibia artery and peroneal artery). The regions were assessed for arterial vascular opacification by two independent researchers on a scale from 0 to 2, with 0 meaning no opacification, 1 meaning partial opacification, and 2 meaning complete opacification of the arteries. Results were analyzed utilizing Fisher's exact test for left and right artery opacification comparison.

With added costs of approximately €66 (approximately \$297), including personnel and materials, and an additional procedure time of 15–20 minutes, successful infusion of contrast mixture was achieved with the enteroclysis pump. Visualization of amount and extensiveness of structures, such as tumors in soft tissues, improved with PMCTA, along with improved identification of thrombi. Partial or complete opacification was measured in 89% of arteries. For arteries with both a left and right component, there was no significant difference between sides.

This study successfully evaluated an inexpensive and easy-to-use method to perform PMCTA for clinical postmortem investigations. This study presents a novel approach to PMCTA, increasing the technical arsenal of the autopsy pathologist and radiologist. With more and cheaper methods becoming available, this could help increase the acceptance of PMCTA by a wider audience.

#### Computed Tomography Angiography, Autopsy, Postmortem



## H8 The Utility of Routine Histological Sampling in the Assessment of Cause and Manner of Death in Medicolegal Autopsies—Fire, Immersion, and Traffic-Related Deaths

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**Learning Overview:** The goal of this presentation is to reveal the value of routine histological sampling in deaths related to traffic, fire, and immersion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing ethical standards and effectiveness and lowering costs.

In 1999, the European (EU) Committee of Ministers recommended histological sampling of “major organs” in medicolegal autopsies.<sup>1</sup> Also because of local routines, histological examination is often routinely included in the autopsy, although the scientific evidence regarding its value is disputed. Previous studies using mixed populations have shown inconsistent results, some highly favorable and others dismissing its utility.<sup>2-7</sup>

In order to allow practical applicable conclusions regarding the necessity of routine histological sampling, it seems necessary to analyze specific Cause Of Death (COD) groups separately. This study focused on deaths related to traffic, fire, and immersion, with the intent of analyzing the value of histology to determine COD and/or Manner Of Death (MOD).

Autopsy protocols, including toxicologic and histopathologic analyses, were acquired from the national database of the Swedish National Board of Forensic Medicine. Study cases were selected based on information available *before* autopsy, suggesting traffic-related, fire-related, or immersion-related deaths. Fifty cases in each study group were selected from the years 2017 and 2018. Any information connected to the decedent, the forensic pathologist, the forensic medicine unit, original assessments regarding COD and MOD, and histological analyses, was masked out. The study was performed in two steps, and there were four reviewers, with two reviewers participating in each subgroup analysis.

**Step 1:** The de-identified autopsy protocols (excluding histological analyses) were sent to two independent, board-certified forensic pathologists who independently reviewed the macroscopic and toxicologic findings. The reviewers determined the COD and the MOD and graded the certainty of their assessments using an incremental scale.

**Step 2:** After reporting the results of Step 1, the reviewers were given access to the histologic analysis in each case, then reevaluated their previous assessments, including the degree of certainty. Any discrepancy between ante-histology and post-histology assessments regarding COD and MOD was analyzed.

Cases were classified on a 1–5 scale as to the extent of agreement ante- vs. post-histology, and the underlying COD was registered in all cases, both ante- and post-histology. Ordered-logistic and logistic Bayesian generalized linear models were used to model the effect of histology on underlying COD, MOD, and agreement.

**Agreement:** In all groups the inter-observer agreement was high both ante- and post-histology. There were minor shifts in agreement in the immersion and traffic-related deaths but none in the fire group. The effect of histopathology on agreement was credibly 0 (-0.4, 0.4 95% Highest Posterior Density Interval [HPDI]) in all groups.

**Underlying COD:** The probability of changing COD for an average observer was small in all groups; in fire cases 0.0038 ( $1.02 \times 10^{-26}$ , 0.016 95% HPDI), in immersion 0.018 ( $9.02 \times 10^{-12}$ , 0.068 95% HPDI), and in traffic cases 0.032 ( $1.38 \times 10^{-13}$ , 0.13 95% HPDI).

**MOD:** The probability of changing MOD for an average observer was small in all groups; in fire cases 0.0038 ( $1.74 \times 10^{-33}$ , 0.015 95% HPDI), in immersion 0.024 ( $4.08 \times 10^{-16}$ , 0.079 95% HPDI), and in traffic cases 0.013 ( $2.65 \times 10^{-11}$ , 0.043 95% HPDI).

**Certainty of assessment:** Regardless of change in COD and MOD, the change in certainty in COD was highly variable. In fire cases, the probability of change was 0.074 ( $4.25 \times 10^{-7}$ , 0.25 95% HPDI), in immersion 0.74 ( $1.42 \times 10^{-7}$ , 0.26 95% HPDI), and in traffic cases 0.11 ( $1.69 \times 10^{-7}$ , 0.37 95% HPDI).

**Conclusions:** Based on the results, and when considering the economic, ethical, and legal aspects, it is concluded that routine histologic sampling is not mandatory in any of the three study group scenarios. Instead, histologic sampling should be left to the experienced forensic pathologist to decide on a case-by-case basis.

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### Histological Sampling, Medicolegal Autopsy, Cost-Benefit

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### H9 Fibroplasia Ossificans Progressiva (FOP): Could Autopsy Define Syndromic Features?

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**Learning Overview:** After attending this presentation, attendees will better understand this rare genetic disorder, characterized by ossification of soft tissues, and be aware of the most frequently associated causes of death: thoracic insufficiency syndrome, recurrent respiratory infections, and accidental trauma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with the understanding that postmortem investigations could discover additional features, unknown in the clinical setting, improving the knowledge about the pathophysiology of rare diseases. This could directly impact also on earlier diagnosis, prevention of complications, and rehabilitation of patients.

This study describes the autopsy findings in a 27-year-old woman affected by FOP who died after a fall from a stairway. Cardiopulmonary resuscitation was unsuccessfully performed by responders. While the death was suspected to be attributable to traumatic head injury and intracranial hemorrhage, an autopsy was requested due to the unclear circumstances of the fall.

FOP is a rare (1/2.000.000 inhabitants) autosomal dominant disorder resulting in progressive bone formation in soft tissues due to the mutation of the Activin Receptor 1 (ACVR1) gene, which encodes for Bone Morphogenetic Protein type-1 Receptor (BMPRI). The ossification of muscles, tendons, ligaments, and other mesenchymal tissues, in association with congenital skeletal malformations, leads to a severe reduction of joint mobility and therefore to severe disability.

At autopsy, rigor mortis was difficult to evaluate due to FOP-related firm rigidity as the upper and lower extremities were diffusely fixed in flexion. An ogival palate with tooth overcrowding was also seen. There was kyphoscoliosis of the spine and major deformity of the chest wall resembling pectus carinatum. Hallux valgus was also observed.

There was evident trauma of the head, including a laceration of the scalp in the vertex region and a diastatic median skull fracture originating from the vertex and involving the fused metopic suture, crossing the anterior cranial fossa and ending in the sella turcica. Associated diffuse subdural hemorrhage and subarachnoid hemorrhage, especially in the cerebellum and around the brainstem, was evident. There was also a right periorbital hematoma with bruising of the left forearm, the back side of the left hand, and the right leg. Microscopic sections of the brain demonstrated recent cerebral hemorrhage. The spine was intact, whereas ribs were fractured on both sides. Both lungs showed subpleural petechiae, edema, and congestion. Microscopic sections of the lungs showed acute emphysema and intra-alveolar hemorrhage, with areas of edema. The thyroid gland appeared grossly unremarkable, but histologically showed diffuse lymphocytic infiltration and some fields of fibrosis. In a histologic sample of muscular tissue (quadriceps femoris), small areas with necrosis of myocytes and hyperplasia of fibroblasts were seen, indicating an initial thin fibrosis. Also, the tendon of the left pectoralis major muscle was completely ossified.

The cause of death of this young woman was certified as being due to severe head and brain trauma. Joint stiffness could explain the walking disability and thus the fall. The investigation led to the manner of death being certified as accidental. The finding of thyroiditis—although clinically silent—appeared consistent with the other reports and probably correlated to the gene mutation. Even non-affected muscle areas already exhibited microscopic foci of inflammation and degeneration. Further study of these elements could better help define characteristics of FOP syndrome.

#### Fibroplasia Ossificans Progressiva, Thyroiditis, Fibrosis





## H10 A Questionable Cause of Death Alphabet Soup: Anaphylaxis, Bees, Cocaine, Diabetic Ketoacidosis (DKA), Emphysema, Fogger, Hypothermia, Insecticide, and Opiates

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**Learning Overview:** After attending this presentation, attendees will gain a better understanding of the proposed mechanism and etiologies of gastric Wischnewsky Lesions (WLs).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting a case in which a clear cause of death was confounded by various findings via history, forensic autopsy, toxicology, and other testing.

WLs are dark, discrete mucosal lesions 1mm to 4mm in diameter found scattered on the gastric mucosa. Since their discovery, they have been thought to be virtually pathognomonic for hypothermia-related death. More recently, they have been associated with pancreatitis, burns, and DKA.<sup>1-3</sup> Here is presented the case of a diabetic man who was found dead in his home in the wintertime with multiple potential causes of death, including DKA, hypothermia, anaphylaxis, drug overdose, and insecticide poisoning.

A 58-year-old man with a history of hypertension, diabetes, and bee sting allergy was found naked and deceased in his home in December. Found within the home were plastic bags containing a powdery white substance, a room with hundreds of dead yellowjacket wasps, empty cans of insecticide and foggers, and a used epinephrine pen. The home's furnace was working, and the temperature was 69°F. Forensic autopsy revealed multiple healing excoriations of the lower extremity, some of which contained dark, linear foreign bodies measuring 3–4mm in length. Early decomposition was also present, with softening of the internal organs and decomposition fluid within the pleural cavities. The lungs revealed pink-red crepitant parenchyma consistent with chronic asthmatic bronchitis. The stomach contained partially digested food/fluid, and the gastric mucosa contained numerous well-developed WLs. Toxicology testing on urine was positive for alprazolam, morphine, codeine, and benzoylecgonine, while heart blood testing revealed alprazolam (3.2ng/mL), morphine (28.4ng/mL), codeine (587ng/mL), acetone (0.038%), and isopropanol (0.025%). The absence of cocaine and the cocaine metabolite benzoylecgonine in the blood was not unexpected given that these compounds are extensively degraded in unpreserved blood. Serum trypsin was 15.9ng/mL and urine n-methylhistamine was 177mcg/g. Vitreous acetone was 0.062%, vitreous glucose was <2mg/dL, and hemoglobin A1c was 5.9%. Despite the multiple potential competing possibilities, the major contributing cause of death was considered diabetes mellitus with ketoacidosis.

WLs were once thought to be pathognomonic for hypothermia, with one study suggesting 88.3% specificity.<sup>4</sup> While the exact mechanism is unknown, current hypotheses involve gastric gland hemorrhage secondary to hypothermic stress, metabolic derangement, and coagulopathy.<sup>5</sup> In particular, coagulopathy is thought to be a potential link between hypothermia, DKA, and WLs, as hypothermia can have an anticoagulative effect that is enhanced by acidosis.<sup>6-7</sup> While the presence of WLs was suggestive of hypothermia and/or DKA in the present case, a definitive cause of death was precluded by multiple confounding findings. While the patient's naked state raised the possibility of "paradoxical undressing" often seen in hypothermia, the working furnace and temperature made this less likely. Similarly, while a history of diabetes and vitreous acetone of 0.062% suggested DKA, the vitreous glucose was <2mg/dL and hemoglobin A1c was only 5.9. The history of bee sting allergy, the presence of yellowjackets, and the used epinephrine pen further obscured the picture; however, serum trypsin and urine n-methylhistamine were within reference ranges. The presence of used insecticide cans and foggers raised the possibility of toxicity due to pyrethroids, synthetic insecticides whose popularity have recently climbed due to increasingly restricted use of organophosphates and use as a recreational drug.<sup>8</sup> Additionally, natural disease (hypertension, asthma) and/or drug use (cocaine, opiates) could not be totally excluded as being potentially contributory to death. Ultimately, the presence of acetone and isopropanol, as well as the florid gastric WLs, led to the conclusion that ketoacidosis probably played the most significant role in death. This case represents an excellent example of a situation in which multiple competing potential factors may have contributed to death. Forensic pathologists are well aware of the fact that, in many cases of this type, a definite answer is unable to be determined with certainty.

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### DKA, Hypothermia, Wischnewsky



### H11 The Importance of Dashboard Camera Analysis in Fatal Vehicle-Pedestrian Crash Reconstruction

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**Learning Overview:** After attending this presentation, attendees will be aware of the importance of a comprehensive investigation at the death scene, including the analysis of Digital Video Recorder (DVR) dashboard camera images for the reconstruction of car crashes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the risk and the benefits related to the use of a DVR. Indeed, as demonstrated from the reported case, a DVR may be of the utmost importance to protect the driver against the distortion of facts. On the other hand, DVR devices attract negative attitudes for privacy concerns and are not legal in many states.

A DVR is an onboard camera that continuously records the view through a vehicle's front windscreen and sometimes through the rear or other windows. Moreover, some dashboard cameras (i.e., "dashcams") include a camera to record the interior of the car and can automatically send pictures and video. Some more sophisticated DVRs may even allow for recording of other data, such as acceleration/deceleration (g-force), speed, steering angle, and Global Positioning System (GPS) information.

The case of a vehicle-pedestrian fatal crash illustrating the importance of DVR analysis is herein presented. A car rolled many times over the road. The driver was able to climb out of the car and walked toward the street, but was subsequently hit by a Russian articulated lorry (i.e., a tractor-trailer truck.) An autopsy was performed one day later and demonstrated multiple extensive traumatic injuries, including fractures and lacerations of the head with intracranial hemorrhage. There were also traumatic injuries of the thorax, abdomen, and multiple internal organs. Toxicologic analyses were performed on postmortem blood and urine with results positive for 11-Nor-9-Carboxy-Delta-9-Tetrahydrocannabinol (THC-COOH) in urine and negative for all other tested substances (namely, alcohol and common drugs of abuse.) Based on these results, the death was determined to be attributable to the massive cranio-encephalic trauma, and prior cannabis abuse was suspected. The manner of death was initially hypothesized to be an accident based on the absence of previous suicidal attempts/ideation, and scene investigative findings, including the lack of a sidewalk and the very narrow roadside available for pedestrians on the rural road where the collision occurred. Ultimately, however, the analysis of DVR images clearly identified a deliberate rush at the front of the Russian articulated lorry, leading to the certification of the manner of death as suicide.

While dashcams are gaining in popularity as a method of protection against the distortion of facts, they may also attract negative attitudes due to privacy concerns. Different countries have varying laws on the use of dashcams. As a result, in some nations (e.g., Russia) DVRs are very popular and are widely used as medicolegal evidence, while other countries ban or strongly limit their use due to concerns regarding privacy. As illustrated by the case reported herein, additional evidence derived from DVRs may be of the utmost importance in accurately determining the manner of death and preventing a miscarriage of justice.

#### Forensic Pathology, Dashboard Camera, Traffic Accident



### H12 Computed Tomography (CT) and X-Ray Angiography on a Case of Traumatic Carotid Artery Occlusion Following a Physical Altercation Resulting in Cerebral Infarction

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**Learning Overview:** The goal of this presentation is to serve to heighten the awareness of the setting in which carotid artery lesions could potentially be missed on autopsy. Application of postmortem CT or X-ray angiography can aid in the identification and evaluation of these lesions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness regarding the appropriate process involved in the evaluation of carotid artery trauma and thrombosis due to blunt force injury.

There is a paucity published in the medical forensic literature on cases related to carotid arterial injury. Furthermore, there is a risk that such a finding may be overlooked during postmortem examination, especially in the presence of multiple blunt force injuries, with misinterpretation of findings leading to incorrect cause and manner of death certification. CT and X-ray angiography can aid in the identification of such an injury. This presentation describes such a case, which provoked a brief review of related available literature.

The decedent was a 33-year-old man with no significant medical history who was involved in a physical altercation with another individual and subsequently complained of feeling unwell. Three days later he was found unresponsive at his residence; emergency medical services responded and transported him to a local hospital where he was diagnosed with a large left-sided stroke. An ultrasound revealed carotid artery occlusion. Despite medical interventions, he was pronounced deceased after brain death was confirmed, and after the family elected to remove life support.

At autopsy, the external examination revealed a healing abrasion on the left posterolateral upper neck, and multiple healing abrasions on the chest, back, and extremities. CT and X-ray angiography of the head and neck were performed and showed occlusion of the left internal carotid artery. Internal examination revealed superficial hemorrhage of the left sternocleidomastoid muscle and patchy soft tissue hemorrhage posterior to the left internal carotid artery, the lumen of which showed an occluding thrombus just distal to the bifurcation of the left common carotid artery. There was no histologic evidence of medial dissection. The thrombus extended to involve the left middle cerebral artery with associated cerebral infarction involving the left cerebral hemisphere. The left cerebral hemisphere showed marked edema. Left cingulate herniation and left uncal herniation were apparent, as well as bilateral tonsillar herniation of the cerebellum. The remainder of the autopsy was essentially unremarkable. The toxicology screen was negative for drugs of abuse. The cause of death of this decedent was certified as acute cerebral infarction due to traumatic carotid artery occlusion following a physical altercation. The manner of death was certified as a homicide due to the temporal relationship between the physical altercation and the development of symptoms.

Singh and associates have reported occlusion involving the internal carotid artery and, in a separate case, occlusion involving the common carotid artery.<sup>1</sup> Both cases had presentations that could have led to misinterpretation of medical findings, leading to missed diagnoses of carotid artery injury and occlusion due to trauma. According to Liu et al., blunt carotid artery injury leading to thrombosis and neurologic impairment has an incidence rate of 1%.<sup>2</sup> Biomechanically, they reported such injuries can occur either via direct blunt force injury, hyperflexion of the cervical spine resulting in arterial compression involving the mandible and spine, oropharyngeal trauma, hyperextension of the cervical spine and rotation, and the fracture of the basal skull causing injury of the intracranial segment of the internal carotid artery. In the forensic literature, An reported that fatal thrombosis involving the internal and common carotid arteries can occur in the setting of little-to-no evidence of external or internal injury, as was seen in the presented case.<sup>3</sup>

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#### Blunt Force Neck Injury, Carotid Artery Thrombosis, Traumatic Vascular Injury



### H13 IL-15, CD-15, and Tryptase as Markers of Wound Vitality in Compressed Neck Skin: When Conventional Macroscopic and Histological Findings Fail

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**Learning Overview:** The goal of this presentation is to expose two cases of hanging suicide in which autopsy and conventional histology were not conclusive in determining the vitality of the ligature mark by analyzing and drawing attention to the use of an immunohistochemical investigation, especially regarding IL-15, CD-15, and tryptase.

**Impact on the Forensic Science Community:** Though most hangings are suicide, homicide and subsequent hanging of the victim are described. This presentation will impact the forensic science community by presenting two cases in which autopsy and conventional histology were not conclusive in determining the vitality of the ligature marks, while immunohistochemistry techniques provided a substantial contribution to the ligature marks vitality estimation.

In forensic practice, it is necessary to distinguish between hanging and simulated hanging. To differentiate between these two occurrences, forensic pathologists have to distinguish antemortem from postmortem ligature marks. Conventional macroscopic and histologic findings may be unreliable; this particularly applies to soft ligature marks, where vital tissue reaction signs are often deficient or absent. Hemorrhagic infiltration is typically considered to be evidence of vital reaction, but Red Blood Cell (RBC) extravasation can also occur after circulatory arrest. The presence of inflammatory cells is the only solid standard histologic finding indicating antemortem origin, and immunohistochemistry is the method of choice to help assess for this in forensic pathology.

Two cases of hanging are presented herein in which the macroscopic findings and conventional histology were not sufficient to enable discrimination between antemortem versus postmortem ligature marks.

**Case 1:** A 29-year-old man was found hanging partially suspended by trousers tied to the bars of the armored door of his cell on the same night he was incarcerated. His cellmate declared that he had not noticed anything. Crime scene investigation was conducted with the presence of the forensic pathologist; there was a small skin abrasion of the left side of the neck (i.e., a barely defined ligature mark only on the left side of the neck). Simulated hanging was suspected. Autopsy external examination revealed conjunctival petechial hemorrhages. Two parched skin abrasions were observed in the frontal and lumbar regions; others skin abrasions were detected on the hands. Internal examination of the neck showed small hemorrhages of both thyrohyoid muscles and of the thyroid cartilage. No fractures were found. Histologic hematoxylin- and eosin-stained sections showed scant erythrocytes in the subcutaneous tissues of the left site of the neck; no others evidence of vital reaction was noted. In order to thoroughly evaluate the case, immunohistochemical investigation of neck skin samples was performed utilizing antibodies against IL-15, CD-15, and tryptase. A positive reaction was achieved for all the antibodies used. The results of the forensic examination, together with immunohistochemical investigation, confirmed the vitality of the skin wound reaction. The cause of death was certified as mechanical asphyxia due to suicidal hanging.

**Case 2:** A 61-year-old man was found hanging by two belts attached to a shower box. The ligature was removed by his brother, who called the police. The autopsy was performed the next day. Two ligature marks, horizontally oriented, were present above the level of thyroid cartilage. There were no petechial hemorrhages. Dissection of the neck showed only limited extravasation of blood in the suprahyoid muscles. The laryngo-hyoid apparatus was intact. Histologic examination of the skin of the ligature furrow did not show the presence of erythrocytes in the tissues, so immunohistochemical studies were performed utilizing antibodies against tryptase and IL-15. Positive staining was observed in the sample, confirming a vital tissue reaction within the ligature mark. The death was attributed to suicidal hanging.

Presented here are two cases in which autopsy and conventional histology were inconclusive in determining the vitality of ligature marks, but immunohistochemistry provided substantial contribution to wound vitality estimation. Tryptase, IL-15, and CD15 can prove to be useful and reliable forensic studies for determining vital tissue reaction in ligature marks.

#### Hanging, Vitality, Immunohistochemistry



### H14 Where is the Neoplasm? The Postmortem Diagnosis of Intravascular Large B-Cell Lymphoma (IVLBCL)

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**Learning Overview:** The goal of this presentation is to share the study results that demonstrate that the best instrument to make a diagnosis is represented by immunohistochemical staining of a tissue specimen (with a specific panel made of CD20 and CD45), while instrumental exams (Computed Tomography [CT], Magnetic Resonance Imaging [MRI], Positron Emission Tomography [PET], and biopsy) exhibit a high number of false negative results.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by how this results in a valid aid for the pathologist when approaching an extremely rare disease, such as IVLBCL.

IVLBCL is a rare non-Hodgkin extranodal B-cell lymphoma that occurs with a prevalence lower than 1%. It has an aggressive clinical behavior and it is characterized by the presence of malignant lymphoid cells within the blood vessels, more specifically capillaries. Making the diagnosis can be challenging because of the heterogeneous and non-specific presentation of the disease due to the generalized organ dysfunction secondary to vascular obstruction; neurological signs and symptoms are the most common presentation of the disease. Clinical diagnosis is based on imaging (CT scan, MRI), peripheral blood smear, cerebrospinal fluid cytology, biochemical examination, and bone marrow biopsy results. Nevertheless, diagnosis may be difficult and literature suggests that very often the disease is recognized only after autopsy examination.

The case presented herein is that of a 50 year-old man with a history of myeloradiculitis hospitalized for paraparesis. The CT showed no evidence of lesions or other altered findings. Lumbar-spine MRI showed conus medullaris swelling between D11 and L1 and vasogenic edema of anterior and posterior cords. Although PET imaging showed an increase uptake in the adrenal glands and a focal area within the mesenteric adipose tissue, both serologic exams and bone marrow biopsy were negative. Spinal cord biopsy showed necrotic nervous tissue with low CD68+ macrophage infiltrates, reactive gliosis, and negative staining for MAP2. After two months, the patient died of respiratory failure.

Gross autopsy examination was negative, apart from findings suggestive of pulmonary infection and reduced consistency of the spinal cord. Examination of histologic samples from the brain, heart, lung, and liver tissue showed collections of atypical cells with enlarged, pleomorphic, hyperchromatic nuclei within blood vessels and interstitium as well as lymphocyte infiltration. Immunohistochemical examination was performed evaluating for the following markers: cytokeratin, CD3, CD20, CD45, PDL-1, CK20, CK7, MCK, and TTF1. A strong intravascular positivity for CD45 and CD20 was found. In sum, these findings would support a B-cell lineage for the neoplastic cells and the postmortem diagnosis of intravascular large B-cell lymphoma.

This case report demonstrates that the most reliable means of making the postmortem diagnosis of IVLBCL is via immunohistochemical staining of tissue specimens (with a specific panel including CD20 and CD45). Other techniques such as radiologic studies (CT, MRI, PET) or bone marrow biopsy show a high number of false negative results.

#### **Intravascular Large B-Cell Lymphoma, Postmortem Diagnosis, Immunohistochemistry**



### H15 Numerical Postmortem Interval (PMI) Estimation Streamlined for Forensic Practice: Combining Photogrammetry, Thermal Imaging, and Computed Tomography (CT)

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**Learning Overview:** After attending this presentation, attendees will have learned how computational algorithms and advanced imaging techniques can be combined to streamline numerical postmortem temperature-based PMI estimation for forensic practice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting, for the first time, an easy-to-use, accurate, and flexible method for temperature-based PMI estimation, potentially replacing the frequently inaccurate current gold standard.

The PMI represents crucial information in forensic investigations and can aid in the reconstruction of the timeline of events. Currently, this information is either guessed at or provided by an empirical model (Henssge's nomogram) based on a set of rectal temperature measurements performed under standardized experimental conditions. In practice, many forensic cases strongly deviate from these standardized conditions, rendering the model's PMI predictions largely inaccurate. A more rigorous and versatile approach to temperature-based PMI estimation was previously developed combining a comprehensive numerical (finite-difference) thermodynamic model and exterior (skin) temperature measurements. In this approach, a discrete (cubic) spatial grid is used to generate a 3D, computational representation of the body and its surroundings, enabling calculation of the heat exchange between the two. Here, this heat exchange is modelled as a combination of heat conduction, convection, and radiation; the body is approximated as an assembly of cones, ellipsoids, and cylinders (composed of adipose and non-adipose tissue) the proportions of which are dictated by standardized anatomical measurements (e.g., length of the arms and legs, as well as the circumference of the head, torso, upper arms, and wrists). Consequently, body posture, body dimensions, and environmental variables, such as contact surface, (partial) submersion in water, and (partial) coverage by clothes, are all readily included in the model. Knowledge of the thermal properties of the involved materials and timely probing of the heat exchange process (prior to thermal equilibrium) then allows the estimation of the PMI. Indeed, cooling curves measured on deceased human bodies are in close agreement with the model predictions yielding estimated PMIs within, at most, 2.7h of true PMIs between 7h and 20h. As a result, this approach is not only more versatile and widely applicable than Henssge's nomogram, but also outperforms it in terms of accuracy of the predicted PMI.

Despite the predictive capabilities of this approach, its laborious anatomical measurement routine renders application in forensic practice challenging. To address this challenge and streamline this technique, this study introduced photogrammetry-based generation of the model body. Here, the 3D shape of the body is reconstructed from a set of 2D images. This 3D information then serves to simultaneously render the dimensions and the posture of the model body in a robust and rapid manner. Furthermore, photogrammetry allows highly accurate spatial co-registration of measured and simulated skin temperatures through the use of coded imaging targets. This not only increases the PMI estimation accuracy, but also enables the integration of thermal imaging by the same token. As a result, this integrated approach requires minimal user input and physical contact. Besides accurately modelling the geometry of the body and its surroundings, it is crucial that the model's material composition closely resembles reality to ensure accurate simulation of the temperature evolution. An important factor in this effort is the body fat percentage, which was previously estimated using the United States Navy circumference method. In order to model the body composition more accurately, this study utilized PM CT scans to derive the 3D adipose tissue distribution that then served in the thermal property assignment for the model body. Similarly, this tomographic information also allows the modelling of body cavities filled with air (or other materials). Here, this presentation demonstrates the successful integration of co-registered photogrammetry, thermal imaging, and CT scans within a previously developed and validated finite-difference framework for numerical PMI estimation. Together, the versatility, limited required user input, and sample contact constitute the streamlined nature of this integrated approach and, consequently, establish its potential for future use in forensic practice.

**PMI, Thermodynamic, Numerical**



### H16 When Hidden Elder Abuse Leads to Death: An Analysis of Casework

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**Learning Overview:** After attending this presentation, attendees will understand the fatal consequences of elder abuse and its different forms, the current situation of underestimation of the problem, and the need to develop effective prevention programs based on the understanding of risk factors.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an analysis of six forensic cases in which the diagnosis of elder abuse was only raised postmortem after an accurate investigation of the scene, of the circumstantial data, and of the autopsy evidences.

According to the World Health Organization (WHO), elder abuse is defined as “a single, or repeated act, or lack of appropriate action, occurring within any relationship where there is an expectation of trust, which causes harm or distress to an older person.” It is currently considered a major public health issue, even though underestimated and underreported, with identification remaining a challenge, especially in the health care context. Moreover, specific “gold standard” diagnostic criteria/markers are still lacking. Therefore, the identification of elder abuse may occur only after the death of the victim. In the present study, six forensic cases of death where elder abuse was suspected were analyzed. In these cases, elder abuse was not always directly related to death, but often contributed to the context in which death occurred. The determination of elder abuse was made based on the combination of all the findings available from the autopsy, the scene analysis, and the circumstantial data.

Case 1 was the homicide of an 85-year-old woman due to strangulation by her cohabiting mentally ill son; the cachectic corpse and the presence of untreated bedsores indicated long-lasting neglect that was unrecognized and unreported. Case 2 was a 65-year-old man who lived alone and died due to winter exposure in a home with broken windows and no heat; self-neglect contributed directly to death. Case 3 was an 81-year-old man sharing an apartment with his mentally ill son; although his death was attributed to natural causes, a context of neglect was evident as he showed postmortem signs of rat injuries to his legs. Case 4 was a 73-year-old completely isolated woman who died of natural causes at home, but was found with her face and neck disfigured by her dog; the modality of death was based on the isolated vulnerable status of the elderly woman. Case 5 was that of a cohabiting mentally ill son who kept the body of his 92-year-old mother at home for about two weeks after her natural death; elder abuse was determined due to lack of social and health services, despite the presence of risk factors. Case 6 was an 86-year-old man who suffered a femur fracture while trying to escape from a long-term care facility where residents were maltreated. The fracture led to hospitalization and death: mistreatment of residents by staff members in these facilities occurred with sufficient frequency to be of concern to health professionals.

In all the cases, the common denominator was the fact that the diagnosis of elder abuse or neglect was only made postmortem based on scene investigation, autopsy, and analysis of all the circumstantial data. The gathering of this information allowed for recognition of the presence of risk factors not previously assessed or reported by anyone. Currently, there are no specific markers of elder abuse, but history and background information together with the autopsy findings indicative of neglect and/or evidence of physical injuries may lead to diagnosis and show that the early recognition of risk factors could help in prevention and treatment approaches.

#### Elder Abuse, Autopsy, Risk Factors

### H17 A Corpse in a Suitcase: A Case of Strangulation

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**Learning Overview:** The goal of this presentation is to report the case of a 54-year-old woman strangled and hidden in a suitcase. A careful crime scene investigation, an external examination, and a complete autopsy with the histological study of skin specimens to determine the vitality of injuries are mandatory to establish the differential diagnosis between the different types of asphyxia deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the usefulness of a multidisciplinary forensic approach by the crime scene analysis, external examination, autopsy, and histological/immunohistochemical and toxicological investigation in cases of strangulation.

A 54-year-old woman was found lifeless inside a suitcase on a cliff near a mountain road in southern Italy. The prosecutor's officer alerted the forensic pathologist, and a detailed crime scene investigation was performed. The body was found curled in a left lateral position with the head flexed. The right upper limb was abducted anteriorly, flexed and intrarotated, and the left upper limb was abducted and flexed; the flexed left hand rested on the left knee, and the lower limbs were flexed to the maximum degree, with feet twisted and placed right on the left. The head was wrapped by a jacket. The cadaveric temperature was 19°C. Rigor mortis was present in all the examined anatomical regions and hypostasis was fixed.

At autopsy, external examination revealed widespread deepithelialized areas and purplish areas on the face and the right anterior neck. The fifth metacarpal bone of the right hand demonstrated a deepithelialized area with the shape of a dental arch. The victim had multiple petechiae and areas of hemorrhage in both sclera and conjunctivae. Many reddish, half-moon-shaped deepithelialized areas were founded on the anterior side of the neck. During internal examination, the tissues of the neck were dissected in layers, revealing diffuse hemorrhagic infarction of subcutaneous tissues. Autopsy also showed airway hemorrhagic infarction, especially in the thyroid cartilage region. Diffuse subarachnoid and intraventricular hemorrhages and pulmonary petechiae were found as well. The analysis of the other organs was unremarkable. Hematoxylin and Eosin (H&E) stained histologic sections revealed polyvisceral stasis, extensive cerebral subarachnoid and intraventricular hemorrhages, pulmonary edema, and acute emphysema. An immunohistochemical investigation of skin samples was performed utilizing antibodies anti-CD15, anti-tryptase, and anti-IL15, which appear to be reliable markers of vital tissue reaction. Immunohistochemistry showed a patchy dermal strong positivity of CD15, tryptase, and IL-15, confirming a vital reaction within injured tissue. Blood toxicologic examination was negative for alcohol or drugs.

Signs of asphyxial death (i.e., petechiae, areas of hemorrhage in both sclera and conjunctivae, pulmonary edema, and acute emphysema), half-moon-shaped deepithelialized areas on the anterior side of the neck, the airway hemorrhagic infarction, and the acute vital reaction within injured tissues together allowed for confirmation of the cause of death as homicide by strangulation.

### Strangulation, Homicide, Corpse Concealment





### H18 The Analysis of Head Injury in the Evaluation of Manner of Death: A Forensic Case Series and a Review of the Literature

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**Learning Overview:** After attending this presentation, attendees will be able to describe the role of a head injury pattern.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how head injuries show typical patterns according to the mode of death.

Head injuries are very common in forensic pathology. Injuries are classified by mechanism, morphology, and severity. According to mechanism, the injuries are open or closed. According to morphology, there are concussions, hematomas, contusions, and cerebral hemorrhages. Severity is assessed through the symptoms. In forensic pathology, head injuries are important in determining the cause and manner of death. They may differ in cases of murder, accident, work-related, or natural deaths; the pattern shows typical features according to the manner. Further, in each case, the analysis of head injuries provides useful information. Described herein are the patterns of head injuries that occurred in four forensic cases.

A woman was found murdered. The autopsy showed 20 different open head injuries. Each injury had indented edges with rounded and convex features. The lesions were overlapped, with depressed skull fractures. The morphology of the edges suggested that the perpetrator hit the woman's head repeatedly using a circular metallic tube. This dynamic was confirmed by the murderer.

In the second case, a man of about 50 years of age fell from a height during work. There were multiple bruises on his head. The autopsy showed major head injury with cerebral hemorrhage, subarachnoid hemorrhage, and multiple right frontal-parietal-temporal region fracture lines. The man was not wearing either a helmet or body harness.

In the third case, a man died in a road traffic accident as a passenger. The man was found outside the car with a large head injury and exposure of the brain. Head trauma was characterized by a large parietal fracture and laceration of the skin. The car had a broken window (point of impact), and the encephalon residues were also found on the guardrail. The autopsy showed that the man was not wearing a seat belt.

In the last case, a man was found dead in the countryside. The man had a bruise on the left parietal region, with red lividity on the thorax and neck. The autopsy revealed no fractures nor brain injuries and proved that the man died from heart failure.

According to a literature review, 39% of deaths related to head injuries are accidents, 43% are homicides, and 17% are undetermined. In work-related deaths, head injuries are the most common finding. There are no data about natural deaths.

The review showed that multiple overlapping injuries with subdural hemorrhage and depressed skull fractures are a typical pattern seen in homicide by blunt trauma. Analysis of wound edges is crucial for determining the weapon, while the evidence of skull fractures and brain injury is useful for estimating the strength of the strokes inflicted. The dynamics can be reproduced using experimental models, then compared with the suspects' witnesses.<sup>1,2</sup>

In cases of accidents, it is common to find multiple, extended, comminuted cranial fractures involving the base of the skull, with Le Fort fractures of frontal facial bones. These findings must be compared with the data collected on judicial inspection and by analyzing the role of the victim (driver, passenger, pedestrian). Also in accidents, injuries are crucial for reconstructing the dynamics of the impact.

In work-related deaths, head injuries are, in most cases, a consequence of fatal falls from a height. Therefore, the severity depends on the height. Head injury pattern can prove the lack of adequate safety measures. In the case reported, the autopsy findings proved the worker did not wear a helmet or body harness. The head injury pattern was subsequently reviewed/utilized in court proceedings.

In literature, there are no data about natural deaths. In the case reported, only bruises related to a fall were found, with no signs of cranial fracture or brain injury. In cases of unilateral, single, and minor head injuries, investigating for typical signs of natural events, such as red hypostasis on the neck and thorax, is suggested. Autopsy can solve the doubtful cases and establish the cause of death.

This study underlines that head injuries are relevant forensic evidence. The pattern changes depending on the case, but it shows recurring features depending on the mode of death. Therefore, this study proposes always performing an accurate investigation of the pattern. The circumstantial data and autopsy are necessary for a precise reconstruction of the event.

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#### Forensic Sciences, Head Injury, Pattern



### H19 The Anesthesiologist's Responsibility in Intracranial Placement of the Nasogastric Tube (NGT): A Case Series and Literature Review

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**Learning Overview:** The goal of this presentation is to share the dangers of NGT misplacement and penetrating the cranial cavity as the consequence of a perforated ethmoid-sphenoid floor. Medical past history and patient records need to be analyzed before any clinical decision, even in an emergency setting, and correct positioning of the NGT ascertained with X-ray or a dedicated pH indicator paper.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of the risks of an apparently simple and uneventful medical procedure, which nevertheless could cause serious, sometimes fatal, complications.

This presentation aims to share with forensic science community the risk and consequences of NGT misplacement, including perforation of the ethmoid-sphenoid floor with subsequent penetration of the cranial cavity. NGTs have a key role in the management of hospitalized patients, especially critically ill ones. Numerous cases of inadvertent penetration of the intracranial compartment by an NGT in the setting of skull-based trauma and/or surgery have been reported. Blind endonasal procedures are particularly at risk of being associated with severe complications. Inadvertent insertion of NGTs into the cranial cavity has also been reported in non-traumatic settings, especially when certain congenital or acquired anatomic anomalies are present. Marked nasal septal deviation, underdevelopment of nasal turbinates, and high-grade pneumatization of the paranasal sinuses are examples of anomalies that may predispose to incorrect positioning of a NGT.

The first illustrative case presented here is that of a psychiatric patient with a history significant for the occasional use of hashish and other drugs and an unknown lesion of the ethmoid sinus. One morning, he was found unconscious in the bathroom of the hospital. Due to suspicions of drug use and a positive response to opioid antagonist administration, an NGT was placed. The second case is that of a patient with intracranial bleeding following pituitary macroadenoma transphenoidal surgery. During emergent decompressive craniotomy, the anesthetist placed an NGT. Retrospective analysis of two such cases provides an important opportunity to assess the effect of a malpositioned NGT from a medicolegal point of view, educate regarding evidence-based methods for device insertion, and overall reduce risks.

Before any invasive procedure (even if emergent), it is advisable to take time to review patient history and medical records. Most importantly, the clinical situation should be carefully assessed for factors such as impaired consciousness, anatomical facial abnormality, and head-facial injury. The second evaluation that must always be carried out is to determine whether nasogastric feeding is appropriate for the patient, and the rationale for any decision must be recorded in the patient's medical notes. To prevent malpositioning of an NGT, a radiological control must be performed; if this is not available, pH indicator paper should be used to test for human gastric aspirates. If there is insufficient support to confirm NGT placement, placement should be delayed until that support is available (unless clinically urgent.) These procedures are useful in order to avoid subsequent damage to anatomic structures; however, they are futile if intracranial insertion of the tube has already caused brain injury.

In the first presented case, the patient survived the malpositioned NGT without neurological sequelae, while in the second case the patient died due to intracranial hypertension. From a medicolegal point of view, the anesthetist's decision to place an NGT in the first case was not deemed wrong due to the suspected drug use; the public prosecutor thus acquitted the anesthetist of criminal negligence. In the second case, however, the decision to insert an NGT was deemed incorrect due to the previous known surgical procedure; the public prosecutor thus convicted the anesthetist of incompetence and imprudence. In conclusion, though typically uneventful, NGT positioning can rarely be complicated by misplacement. Past medical history and patient records should be reviewed before making the clinical decision to insert an NGT—even in an emergent setting. Correct NGT positioning can be ascertained via X-ray or dedicated pH indicator paper.

#### Nasogastric Tube, Intracranial Placement, Anesthesiologists' Responsibility

## H20 Powder-Free DNA Extraction Protocol From Bones and Teeth

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**Learning Overview:** After attending this presentation, attendees will be aware of how to better choose a protocol to extract DNA from bones and teeth. Most importantly, attendees will be encouraged to conduct the same modifications to the pulverization protocols they currently use, which may improve DNA typing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping forensic practitioners in extracting DNA of enough quality and quantity from decomposed teeth and bones to obtain a full Short Tandem Repeat (STR) profile. In addition, the performance of the presented protocol was shown to be superior to a validated commercial protocol in an actual forensic case.

DNA analysis is considered the gold standard in the identification of human remains. In that context, the aim of genetic analysis is to obtain a DNA profile from a deceased person to compare to those of putative relatives or personal objects. However, DNA must first be extracted from a biological substrate in order to be analyzed. Often, hard substrates, such as bones and teeth, are sampled from decomposing corpses, especially in cases of long postmortem intervals. That is because hard tissues usually preserve DNA better than soft tissues. Nevertheless, there is no consensus in the forensic community regarding which method should be used for extracting DNA from skeletal samples. There is a widely adopted practice only regarding sample preparation, which involves pulverization of bone pieces or teeth.<sup>1</sup> As an example of this, the commercial kit QIAamp® DNA Investigator by QIAGEN® provides a validated protocol for use with bones and teeth that require sample pulverization. It must be noted, however, that powdering mineralized tissues has setbacks, which has led to some researchers advocating for less destructive and powder-free protocols.<sup>2,3</sup> For example, pulverizing bone and teeth precludes future morphological analysis, as well as destroys unique specimens. Furthermore, bone and tooth powder are affected by static from the contact with plasticware. That phenomenon may attract particles to the outside of microtubes, promoting sample loss and contamination. In fact, the commercial protocol mentioned above has been used in the laboratory and has failed to provide a DNA profile using bones and teeth collected from real cases. Considering those and other results published in the literature, it appears that sample powdering may negatively affect the integrity of DNA molecules.<sup>4</sup>

Therefore, the goal of this study was to assess the performance of a modified protocol for DNA extraction from bones and teeth that does not require sample powdering. Premolar teeth and femur diaphysis samples, collected from a human body buried in a cemetery for 46 years, were processed. Two femur samples and a tooth were processed using the standard protocol, while two femur samples and two teeth were processed using the modified one. For the standard protocol, the QIAamp® DNA Investigator kit protocol for bones and teeth was followed. For the modified protocol, whole teeth and femur pieces were individually demineralized in 20mL of EDTA solution (0.5M, pH 8) at room temperature. Teeth crowns were first covered in parafilm, after which the specimens were demineralized for approximately 18 hours. Femur pieces were demineralized for at least three days. After demineralization, up to 200mg of the demineralized portions of the samples were cut off with disposable scalpel blades and processed using the same commercial kit following the protocol designed for “soft” tissues. For teeth, the lower half of the roots were sampled, especially the tips.<sup>5</sup> Extracted DNA was quantified using an in-house quantitative Polymerase Chain Reaction (PCR) assay that targets two human-specific DNA regions, of 67 and 170–190 base pairs. The samples were submitted to amplification of 16 DNA markers using Thermofisher Scientific’s™ NGM™ Select, and detection of DNA fragments was conducted in a Thermofisher Scientific™ 3500 Genetic Analyzer.

The standard commercial protocol studied did not yield detectable quantities of DNA from the femur or tooth samples. On the other hand, the proposed modified protocol provided good enough quantity and integrity of DNA to produce full and concordant DNA profiles. These findings reinforce the need to fully demineralize bone and teeth samples prior to DNA extraction, which is not achieved by the standard commercial protocol used.<sup>6</sup> Moreover, a previously reported protocol that did not pulverize bone samples was also shown to be more efficient than when pulverization was performed.<sup>4</sup> In conclusion, the results of this study are in accordance with other studies, also suggesting that full demineralization of bones and teeth portions may be more efficient than extracting DNA from pulverized bones and teeth.<sup>4,5</sup>

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### DNA, Forensic, Human Identification

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### H21 A Complete Transection of the Aorta During Resuscitative Efforts

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**Learning Overview:** The goal of this presentation is to present an unusual injury sustained during resuscitative efforts and explain the rationale behind the classification of the injury.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating attendees about the characteristics of resuscitative injuries, which will allow scientists to classify injuries appropriately, even when atypical.

A variety of resuscitation injuries and their frequencies have been reported in the literature, including contusion and thermal injury of the chest, sternum and rib fractures, lacerations to underlying organs, and laryngeal and tracheal contusions.<sup>1-3</sup> They are accepted as a risk of potentially life-preserving measures of last resort. On occasion, though, these injuries may be so severe that they would likely prove fatal were the patient to regain a pulse. They also may cause confusion for investigators performing an examination after death.

This report presents a case of a complete transection of the aorta in a decedent who received in-hospital resuscitation, an occurrence that has not previously been reported as a consequence of vigorous resuscitation efforts. In this case, a 56-year-old woman was found unconscious but alive in a vehicle along a roadway with no signs of injury, according to the report by first responders. She was transported to a hospital, where she was admitted to intensive care for ten hours with a differential diagnosis of sepsis or hyperthermia with dehydration before she lost a pulse. Over the course of the next three hours, Cardiopulmonary Resuscitation (CPR) was performed five times before resuscitation efforts were halted and death was pronounced. At autopsy, she had a contusion over the midline of the chest, a sternal fracture, fractures of the first through eighth ribs bilaterally, lacerations of the epicardium and myocardium, and complete transversely oriented transection of the descending aorta posterior to the heart. The surrounding mediastinal soft tissue contained a mild to moderate amount of hemorrhage without dissection planes and without hemothoraces.

Such an injury would not be compatible with life were she to have survived with return of spontaneous circulation, raising questions about the futility of resuscitative efforts for the medical community. For medical examiners, this or similar injury may be incorrectly attributed to forceful deceleration in a different circumstance, especially since the decedent was found in a vehicle. However, the decedent would not have survived 13 hours in the hospital (in addition to the unknown amount of time at the scene) if the injury had occurred around the time that she was found. Additionally, the mild to moderate amount of hemorrhage present in the mediastinum without significant blood in the pleural cavities indicates the injury occurred shortly prior to death, before development of significant bleeding under arterial pressure. Correlation of the autopsy findings with hospital records also provides a way to attribute the injury to resuscitative efforts. While CPR may vary greatly in efficacy and force among those performing it, the reports of prolonged, repeated resuscitation performed by trained health care providers in this case offers a plausible mechanism of injury.

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#### Autopsy, Resuscitation, Artifacts



### H22 A Proposal of a Score to Evaluate Discrepancies Between the Results of External Body Examination and Forensic Autopsy

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**Learning Overview:** After attending this presentation, attendees will be aware that there is a great discrepancy between the results of external body examination and a full forensic autopsy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing a mathematical calculation method in order to standardize the analysis of discrepancies existing between the external body examination and a complete forensic autopsy.

In many countries, such as Italy, there has been a gradual decline of forensic autopsies, mostly due to economic considerations aimed at containing justice departmental spending. This reduction of the forensic autopsy has been replaced by external body examinations without subsequent autopsy. The goal of this study was to evaluate the consequences of this trend by establishing the rate of discrepancies between the results of external-only body examination and full forensic autopsy. Similar studies have been previously performed.<sup>1-3</sup> However, there has been no standardization of the analysis method. Therefore a calculation score is proposed in order to standardize methodology and results.

A retrospective study was carried out on 312 autopsy cases of traumatic deaths performed in the Department of Forensic Medicine and Pathology of the University of Perugia and Terni. The results obtained in this study, based on the scoring system proposed, showed a high discrepancy between the results of the external-only body examination versus the full autopsy. The discrepancy was absent in a very small number of cases, for which the external examination alone was exhaustive; it was high, however, in the majority of cases, in which the autopsy was shown to be fundamental in establishing the correct diagnosis of death. Overall, these study results did not support replacing the forensic autopsy with external-only body examinations; rather, they reinforced the fundamental role of autopsy as a gold standard to accurately assess cause and manner of traumatic deaths. Moreover, autopsy, as it identifies a greater number of traumatic injuries, provides much critical data useful for reconstructing scene event dynamics.

The present study conclusions are in agreement with forensic recommendations in the general literature that advise against carrying out an external cadaveric examination alone. External-only examination without autopsy has been shown insufficient in the majority of cases to determine cause of death or obtain other critical information necessary for the purposes of justice. The systematic introduction of a standardized scoring algorithm for trauma in postmortem examinations will further strengthen the leading role of autopsy, helping to create a common language for all aspects of fatal trauma research.

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#### Autopsy, External Examination, Discrepancies



### H23 Immunohistochemical Detection of Fibronectin, P-Selectin, FVIII, HSP-70, and MRP-8 in the Skin of Ligature Marks of Suicidal Hangings

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**Learning Overview:** After attending this presentation, attendees will understand that the determination of the vitality of skin injuries is one of the most central research areas in forensic pathology because it is often necessary to discriminate antemortem wounds from postmortem damage. Typical is the case of hanging in which real hanging has to be distinguished from postmortem suspension of a body. Despite the fact that researchers have intensively investigated this topic in the past, reliable ancillary testing methods are still lacking.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance of the identification of early markers of tissue vital reaction in cases of hanging.

**Materials and Methods:** Fifteen human skin samples from ligature marks and 15 uninjured skin samples from deaths by suicidal hanging were analyzed (11 males and 4 females, mean age of 43.46 years). Fifteen skin samples from ecchymoses in victims of homicide with a short survival interval (9 males and 6 females, mean age of 43.26 years) were studied as control group. Sections were stained with hematoxylin and eosin and were also processed for immunohistochemistry to detect the expression of fibronectin, p-selectin, Factor VIII (FVIII), Heat Shock Protein-70 (HSP-70), and Myeloid-Related Protein 8 (MRP8.) Immunohistochemical reactions were classified semiquantitatively. For statistical analysis, Student's *t*-test was performed and a level of  $P < 0.05$  was considered as statistically significant.

**Results:** Fibronectin—In ligature marks, fibronectin was significantly less expressed compared to ecchymoses. Its expression was similar in hanging marks and uninjured skin. P-selectin—In both ligature marks and ecchymoses, the expression was significantly increased in the endothelial cells and in the perivascular spaces compared to uninjured skin. FVIII:—In both ligature marks and ecchymosis, expression of FVIII was significantly decreased in the epidermis compared to uninjured skin. In the epidermis of ligature marks, FVIII was significantly less expressed compared to ecchymoses. HSP-70—In the epidermis of both ligature marks and ecchymoses, the expression was decreased compared to uninjured negative controls. In the epidermis of ligature marks, the expression was significantly lower compared to ecchymoses. MRP8—In both ligature marks and ecchymoses, the expression of this factor was significantly increased in blood vessels of dermis and hypodermis and also in the hemorrhagic areas compared to uninjured skin.

**Discussion and conclusions:** In uninjured skin, fibronectin is physiologically expressed in the epidermal basement membrane, around skin appendages, and in blood vessels. In the ligature marks, it is just a little more expressed in the hemorrhagic areas, so its detection is not useful in the determination of vitality. P-selectin plays a role in the early binding of leukocytes to endothelium during inflammation and in the recruitment of platelets at areas of vascular injury. In ligature marks, it is extensively more expressed compared to uninjured controls so it could be useful in the determination of vitality. FVIII is a blood coagulation factor that mediates the adhesion of thrombocytes to subendothelial connective tissue. In uninjured skin, it is expressed in the epidermis and in the blood vessels. The decreased expression of FVIII in epidermis of ligature marks could be due to the compression of the skin that reduces the diffusion of the factor in the epidermis; the marker is instead more expressed in the areas of dermal hemorrhage, and it could be useful in the determination of vitality. HSP-70 play an important role in cell responses to stress, and its expression is very high in epidermis of uninjured skin. Mechanical injury due to hanging can reduce the expression of this marker in the epidermis, so it is valuable in the study of vitality of ligature marks. Finally, MRP8 is an early marker of inflammation and because of its increased expression, it could be useful in the study of vitality of ligature marks.

In conclusion, the results of this study show that immunohistochemical study of early inflammatory and coagulation factors could be valuable to determine the vitality of ligature marks. In particular, the combined analysis of p-selectin, FVIII, HSP-70, and MRP-8 can be considered for this purpose while fibronectin seems not to be useful.

#### Suicidal Hanging, Vital Wounds, Immunohistochemistry



### H24 A Metabolomic Profile of Aqueous Humor in a 24-Hour Period After Death: An Animal Model for Postmortem Interval (PMI) Estimation

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**Learning Overview:** After attending this presentation, attendees will understand the potential role of metabolomics in estimating PMI through the study of aqueous humor.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing one more tool in the investigation of PMI.

The estimation of the time since death or PMI remains a major challenge in forensic pathology. Several forensic laboratories are involved in ongoing efforts to characterize and validate an objective analytical method for this purpose. Besides traditional methods based on macroscopic corpse modification, different analytical procedures have been recently proposed for this aim. These include the evaluation of messenger RNA (mRNA) or DNA degradation in postmortem tissues, human tissue transcriptomes, and gene expression patterns, muscle protein degradation based on protein or peptide signatures, the estimation of the hypoxic inducible levels of vascular endothelial growth factor, and assessing postmortem biochemical changes in body fluids (e.g., blood, synovial, pericardial, and cerebrospinal fluids.) Ocular tissues and fluids—especially vitreous humor— have been frequently chosen as reference biological samples due to being more anatomically isolated. Recently, metabolomics have shown to be a potential tool to investigate the time-related postmortem metabolite modifications in animal models. While traditional techniques for PMI estimation are quite subjective in nature, and other proposed methodologies are based on the estimation over time of a single to a few parameters that are potentially prone to the influence of intrinsic/extrinsic factors, the analysis of metabolomic modifications, relying on multiple metabolites/biomarkers quali-quantitative changes, shows a greater predictive power. Here is proposed, for the first time, the use of a  $^1\text{H}$  NMR metabolomic approach for the estimation of PMI from Aqueous Humor (AH) in an ovine model.

A total of 59 AH samples were collected at different PMI (spanning from 118 up to 1,429 minutes, at a 60-minute pace). Thirty-eight (38/59) were used for the training set, while the remaining 21 were employed as test set.  $^1\text{H}$  NMR experiments were performed, and the spectral data was analyzed by multivariate statistical tools. Exploratory data analysis was performed by Principal Component Analysis (PCA) to discover outliers and specific trends in the data. Thus, supervised data analysis based on Projection to Latent Structure regression (PLS2) was applied to evaluate the effects of PMI on the metabolomic profiles of the collected samples. A multivariate calibration model was built to estimate PMI on the basis of the metabolite content of the samples. The model was validated with an independent test set, obtaining a prediction error of 59 minutes for PMI less than 500 minutes, 104 minutes for PMI from 500 to 1,000 minutes, and 118 minutes for PMI greater than 1,000 minutes. During the first 1,000 minutes, lactate was accumulated and strongly influenced the sample distribution along PC1. Early PMI samples were characterized by high levels of leucine and isoleucine, arginine, and lysine, while late PMI samples (>1,000 minutes) showed high levels of taurine, succinate, and choline. Moreover, the metabolomic approach suggested a picture of the mechanisms underlying the postmortem biological modifications, highlighting the role played by taurine, choline, and succinate. The time-related modifications of the  $^1\text{H}$  NMR AH metabolomic profile in the first 24 hours after death seem to be encouraging in addressing the issue of a reproducible and robust model to be employed for the estimation of PMI.

#### Metabolomics, Aqueous Humor, PMI Estimation



### H25 Muscle Protein Degradation in Postmortem Interval (PMI) Estimation: Recent Accomplishments and Current Challenges

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**Learning Overview:** After attending this presentation, attendees will better understand the current state of the art and a specific international collaboration project on postmortem protein degradation for use in PMI estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that time-since-death estimation is a crucial topic in forensic routine, yet currently applied methods are often insufficient or produce unsatisfactory outcomes. New approaches, such as the one presented, are required to contribute to the methodical spectrum.

**Background:** Estimation of the PMI is one of the most challenging aspects of routine forensic work. Current available methods are often limited to a certain timeframe or may be insensitive due to specific case preconditions or the circumstances surrounding the death. The search for additional methods and respective postmortem biomarkers is, thus, a central topic in forensic science and has become of increasing interest since the rise of sophisticated molecular biology approaches over the past decades. This study proposes a novel approach based on muscle protein degradation that has shown great promise by a first successful application in a forensic case. However, respective impacts of individual and environmental influencing factors reveal an important task that necessarily has to be addressed to achieve significant practical relevance in PMI estimation. Also, the applicability to a large heterogeneity of cases requires an appropriate evaluation of the sufficiency and the limitations of a method.

**Methods:** This study investigated possible metabolic influences, such as injury/regeneration or atrophy, in a rodent model and humans. Variances of protein degradation kinetics in different muscle groups (thigh, calf, and abdominal muscle) in rats and humans were examined. A field and crime scene sampling protocol was implemented. The possible influence of changing weather conditions and insect activity on a pig model were investigated.

**Results:** Analysis of muscle protein degradation represents a promising new tool for PMI estimation and is based on a catabolic process that is well-preserved in different mammalian species. Thus, animal models—when thoughtfully employed—represent valid approaches to analyzing various possible influencing factors. This study successfully implemented field and crime scene protocols and demonstrated that postmortem protein patterns can clearly be distinguished from *in vivo* metabolism.

**Conclusion:** This study reinforces the importance of analyzing factors that may influence biomedical postmortem alterations used to estimate the PMI. It also highlights the advantages and utility of muscle protein degradation analysis as a tool for forensic investigations. Overall, it offers insight into the general requirements of novel approaches for time-since-death estimation and the specific challenges of incorporating muscle protein degradation analysis into routine forensic work.

#### Protein, Degradation, PMI Estimation





### H26 The Importance of Being Earnest: The Role of Autopsy in Preventing Litigation Related to the Management of Liver and Digestive Disorders

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**Learning Overview:** After attending this presentation, attendees will better appreciate the role of clinical autopsy in cases of unexpected death after gastrointestinal surgery or endoscopic diagnostic procedures for the management of liver and digestive diseases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by promoting the clinical autopsy as a transparency enhancer able to identify procedural pitfalls and prevent medical malpractice claims.

In recent years, the assertion that autopsies are unnecessary has been strengthened as a result of advanced antemortem diagnostic testing options that have been deemed sufficient to identify clinically significant pathological conditions and causes of death in most cases. Similarly, there is a certain reluctance on the part of non-pathology physicians to request clinical/medical autopsies due to the fear that diagnostic or therapeutic errors may be detected. Only a few studies have investigated the role of autopsy in litigation prevention and clinical risk management. Similarly, the phenomenon of litigation related to the performance of invasive or surgical procedures in the context of digestive and liver diseases is poorly studied despite the hypothesis of a greater risk for the physicians involved.

The case series presented consists of 17 cases of patients who died unexpectedly following endoscopic or surgical procedures for the diagnosis and/or treatment of digestive and hepatic diseases. In all the cases, a preliminary analysis of the medical records and a consultation with the health professionals involved in the management of the patient was carried out. A complete postmortem examination was subsequently performed in the presence of physicians representing the ward and relatives of the deceased. The autopsy technique was tailored to the clinical characteristics of the patients and the suspected diagnosis. Histologic studies were ordered to complete the autopsy investigations and confirm the diagnosis of death. At the end of the investigations, the results obtained were jointly presented and discussed in the interests of transparency.

Overall, this adopted postmortem investigative protocol resulted in confirmation of the initial clinical diagnosis in only two cases (12%). In eight cases (47%), the autopsy provided formulation of a diagnosis different from the clinical one, while in the remaining seven cases (41%), the postmortem examination was decisive since the clinical diagnosis was previously unknown. In 15 cases (88%), the clinical autopsy made it possible to establish the cause of death and—through the sharing of the findings—was fundamental in the prevention of litigation. In the other two cases (12%), the evidence gathered during the postmortem examination did not prevent litigation but did have a decisive impact in the court trial.

In conclusion, the present case series demonstrates the critical role of the clinical/medical autopsy in the management of deaths of patients admitted to the hospital for liver and digestive disorders. In particular, the added value of postmortem diagnostics lies in the possibility of obtaining a definitive diagnosis (which may or may not be consistent with the suspected antemortem diagnosis) and of generating useful evidence for the prevention of litigation or for the better management of a subsequent court trial.

#### Clinical Autopsy, Digestive and Liver Disease, Claims Prevention



### H27 A Preliminary Assessment of the Persistence of Prostate Specific Antigen (PSA) Transfers Under Various Conditions

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**Learning Overview:** The goal of this presentation is to provide attendees with a better understanding of the persistence of PSA detection in semen stains under various conditions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of the persistence of PSA resulting from different manipulated environmental and transfer conditions.

PSA, also known as p30, is a protein used as an effective marker for the identification of seminal fluid lacking spermatozoa cells. The ABACard® p30 test is extremely sensitive and can be used to detect the presence of the PSA protein at levels as low as 4ng/mL.<sup>1</sup> Most current research surrounding the stability of PSA over time looks at it within the female reproductive tract; however, very little research examines the detection of PSA outside of the human body where other environmental factors can influence its detection.<sup>2</sup>

The conditions examined in this study were dry versus wet transfer-receiving substrates, pH, drying time, and differing semen dilution concentrations. For all variables manipulated, the ABACard® p30 test was used for detection. The reaction environment for PSA was manipulated with differing pH levels, starting from a pH of 7, with detection of the protein still possible, up to a pH of 12. The length of time each sample was allowed to dry prior to testing for PSA did not appear to affect detection; however, the rate of appearance of the test band on the ABACard® was found to be directly dependent on the sample's dilution concentration. Fifteen microliters of semen were allowed to dry on a nitrile glove for varying time periods before being transferred to a cotton swatch with the use of constant pressure upon application. It was found that the transfer stains were typically non-detectable by the ABACard® p30 test, under the dry receiving substrate conditions. When the cotton receiving swatch was treated with a personal jelly lubricant, chosen for its water-based formula, the test was positive regardless of the length of time waited prior to transfer. For the wet and dry receiving substrate conditions, as well as the dilution series, Adobe® Photoshop® was utilized to examine test band intensity on the ABACard® p30 cards from photographs taken under controlled conditions. Wet conditions utilized personal jelly lubricant in an attempt to replicate possible moist environments the protein could be transferred to. It was found that the wet conditions yielded more color intense test bands than the dry conditions. Additionally, the wet conditions seemed to stabilize the detection of PSA at differing dilution concentrations, causing the test to behave more consistently. The differences observed between the wet and dry conditions of test band intensity were found to be statistically significant through *t*-testing ( $p < 0.0054$ ). For the dilution series, it was found that lower concentrations yielded fainter test bands on the ABACard® p30 tests. Based on results obtained, PSA is detectable as well as durable under these different environmental conditions. Further research examining longer time periods under certain conditions, as well as increasing the overall number of replicates, would help to more accurately assess any possible statistically significant differences in the findings.

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#### PSA, Persistence, Transfer

## H28 Recovery of DNA From Washed Bloodstains

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**Learning Overview:** After attending this presentation, attendees will be aware that in the forensic sciences body fluids, such as blood and bloodstains, are frequently encountered in homicides. Such evidence, which is biologically obtained at various crime scenes, plays a strong role with forensic identification in determining whether a suspect or victim has an association with the crime. For this reason, DNA analysis is performed routinely in forensic laboratories from biological materials, such as blood, saliva, semen, and hair, that are collected from crime scenes in order to connect the crime scene with suspects and victims.<sup>1,2</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by relating the importance of obtaining DNA from washed clothes for the purpose of revealing powerful physical evidence after the event. In general, bloodstains are the most common evidence at the crime scene and generally can be detected with the naked eye. For this reason, blood and bloodstains that potentially relate to the crime are usually cleared or washed for the purpose of liberation from evidence related to the incident. As a result of different cleaning methods after the crime, body fluids such as bloodstains exposed to cleaning chemicals cannot be seen by the naked eye. Primarily, researchers suggest that washed bloodstains may not have a sufficient quality of DNA for DNA profiling analysis. However, some studies have shown that bloodstains can be used for DNA analysis even after harsh washing protocols.<sup>2-4</sup>

The goal of this study was to determine the rate of DNA recovery after washing bloodstains on different types fabric at different levels. In this study, a representative crime scene was created and bloodstains were made on cotton and nylon fabrics. After washing with laundry detergent at 40°C, 60°C, and 90°C, visualization and identification of bloodstains was conducted. There have been previous attempts to determine whether the amount of DNA required for forensic genetic identification can be recovered from washed laundry. In parallel with the literature on the subject, the current study determined that DNA recovery is directly related to the fabric type; DNA was lost more from non-absorbent nylon fabric types due to easy exposure to external factors. It was demonstrated that greater recovery of DNA can be obtained from cotton-type fabrics, which are more absorbent. The rates of obtaining DNA from bloodstains on different types of fabric that are washed at different temperatures were also evaluated in this study.

In conclusion, when considering the triangle of the crime scene, the perpetrator, and the victim, obtaining necessary biologic evidence to ensure that justice is served is critical. Even in cases in which bloodstained clothing has been washed, it may still be possible to recover a sufficient quantity of DNA for purposes of DNA profiling analysis, as demonstrated by this study.

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### Forensic Biology and Genetics, Bloodstains, DNA From Washed Bloodstains



### H29 Changes in Receptor Expression of $\sigma$ -1R in the Pineal Gland Related to Different Causes of Death

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**Learning Overview:** After attending this presentation, attendees will understand the importance of improving knowledge regarding pineal gland involvement in response to stress related to the death process and their possible changes related to the different causes of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting: (1) whether the pineal gland undergoes structural changes in relation to different causes of death; (2) if these changes have significant differences; and (3) if it is possible to better demonstrate molecular changes in the gland.

N,N-Dimethyltryptamine (DMT) is a potent psychedelic substance produced in the human body, especially in the pineal gland. It has been proposed that DMT is secreted by the pineal gland in large quantities under extreme stress, such as during dying or for a certain period after death.<sup>1</sup> The  $\sigma$ -1 Receptor ( $\sigma$ -1R), is particularly well-expressed in the pineal gland, is located in the endoplasmic reticulum, and plays an important role in regulating the mechanisms of calcium release through the receptor for Inositol Triphosphate (IP3). DMT is the only endogenous agonist ligand capable of activating this receptor: once activated, DMT binds to the receptor and regulates cellular ionic channels, in particular K<sup>+</sup> channels.<sup>2</sup> The goal of the present study was to demonstrate the impact of different causes of death on the expression of  $\sigma$ -1R with immunohistochemical techniques.

The pineal glands from 48 forensic autopsies were included. The specimens from the following causes of death were examined: hanging, stabbing, fire fatality, sudden cardiac death, hemorrhagic shock, and drowning. All autopsies were performed within 24 to 48 hours after death. Pineal glands obtained from the autopsies were fixed in 4% paraformaldehyde in 0.2M Phosphate Buffer Saline (PBS), dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin. Histological sections (5 $\mu$ m) were processed for  $\sigma$ 1-R immunohistochemistry according to the manufacturer's instruction; sections were deparaffinized in xylene and rehydrated in ethanol. Antigen retrieval was performed with pH 6.0 buffer citrate and endogenous peroxidase blocked with 0.3% Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) in PBS. Primary antibody (1/100 dilution) was incubated overnight at 4°C in a moisturized chamber. The day after, peroxidase-conjugated secondary antibody (1/50 dilution; Pierce anti-rabbit, anti-goat, and anti-mouse) was added and the reaction was visualized with 3,3'-Diaminobenzidine (DAB). Counterstaining was performed in Mayer's hematoxylin. Negative control slices were tested using PBS instead of the primary antibody. Overall, results of this study revealed a different expression of  $\sigma$ 1-R immunopositivity in relation to the cause of death, being particularly evident in hanging, fire fatality, and sudden cardiac death. The results were then analyzed in relation to factors such as sex, age, and timing of the death process.

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#### Receptor Expression of $\sigma$ -1R, Pineal Gland, Different Causes of Death



### H30 Early Myocardial Ischemia: An Immunohistochemical Analysis of Dystrophin and Matrix Metalloproteinase 9 (MMP-9)

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**Learning Overview:** After attending this presentation, attendees will understand that the forensic diagnosis of early myocardial ischemia is based on the knowledge of the immuno-inflammatory pathophysiology and cellular phenomena accompanying cardiac alterations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the usefulness of immunohistochemistry to detect early myocardial ischemic damage through the analysis and comparison of C5b-9, fibronectin, dystrophin, and MMP-9 expression.

Sudden Cardiac Death (SCD) is an unexpected natural death due to cardiac causes that occurs within a short time period in a person without any prior condition that appears to be fatal. Coronary Artery Disease (CAD) and ischemic cardiac damage are the main causes of SCD. In cases of cardiac death arising due to ischemia in which the death occurs within six hours from the onset of ischemic damage, the histologic myocardial changes are not specific and cannot provide clear evidence for the postmortem diagnosis.

Immunohistochemical analysis has been suggested as a technique to help bridge the gap in the histologic diagnosis of early myocardial ischemia and several markers have been analyzed.<sup>1</sup> Thus, knowledge about the chronology of the inflammatory reaction and myocardial tissue response after the ischemic insult become very useful in determining immunohistochemical markers relevant to forensic research.

This study evaluates the expression of dystrophin and MMP-9 in cases of SCD due to coronary atherosclerotic disease both with and without definitive microscopic evidence of myocardial ischemia. These proteins have different roles in cardiac tissue: dystrophin is normally expressed in cardiomyocytes contributing in stabilizing the sarcolemma during cardiac contraction and in the transmission of myofibers contraction force, while MMP-9 is a protease generally associated with degradation and regulation of the extracellular matrix and recently related to regulatory mechanism for precise cellular control of biological processes. Their expression was also compared to C5b-9 complex and fibronectin expression to analyze if markers provided the same or no findings.

Results of this study revealed that dystrophin and MMP-9 show different post-ischemic time-dependent expression as, respectively, depletion of sarcolemmal staining and increasing of interstitial and leukocytes immunopositivity occurs. Dystrophin and MMP-9 seem to be useful immunohistochemical markers for the detection of early ischemic damage. These proteins (and especially dystrophin) show early modification of expression as well as of C5b-9 complex.

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**Myocardial Ischemia, Immunohistochemistry, MMP-9**



### H31 A Case of Suicide by Hemlock Intoxication

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**Learning Overview:** The goal of this presentation is to highlight the death of a 52-year-old Caucasian male due to hemlock intoxication, a rare method of suicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the deadly effects of hemlock intoxication on adults and educating attendees on how to identify possible hemlock poisoning through scene investigation and autopsy and also how to confirm the presence of hemlock in various samples through forensic laboratory testing.

**Introduction:** Poison hemlock (*Conium maculatum*), the infamous plant responsible for the death of Socrates, is toxic to humans and animals. Ingestion has been recorded in the literature, mostly in cases of accidental ingestion due to its similar appearance to other plants. In cases where hemlock led to death, the cause of death is usually associated with respiratory arrest, muscle paralysis, or complications of acute renal failure.<sup>1</sup> While the entire plant is toxic, the most dangerous part is the seeds, which contain the highest alkaloid concentration.<sup>2</sup> It contains eight piperidine alkaloids, with the most potent one being coniine. Coniine is a neurotoxin that disrupts the function of the peripheral nervous system, causing respiratory paralysis. Initial symptoms include nausea, vomiting, abdominal pain, hypertension, tachycardia, tremor, and temperature increase.<sup>1,3</sup> Symptoms progress toward central nervous system depression, coma, muscular weakness and/or paralysis, with respiratory distress.<sup>2</sup> In some cases of hemlock ingestion, renal failure and renal tubule damage have been reported.<sup>3</sup> The concentration of alkaloids found in each plant varies depending on age of plant, season, and precipitation.<sup>4</sup> No antidote exists for hemlock specifically.<sup>2</sup>

**Materials and Methods:** This case involved a well-developed (5 feet, 10 inches; 176 pounds) 52-year-old Caucasian male who committed suicide by ingesting hemlock. The decedent had a history of suicidal ideations, as evidenced most recently by suicidal text messages found on his phone. His text messages revealed symptoms of muscle weakness, difficulty walking, and decreasing consciousness. His text messages revealed that he had recently attempted suicide by hemlock ingestion but had been unsuccessful. He also had a history of tobacco use, anxiety, depression, and attention deficit disorder. Various samples were tested using liquid extraction and solid phase extraction and Gas Chromatography/Mass Spectrometry (GC/MS).

**Results:** Aside from slight atherosclerosis and left and right ventricular hypertrophy, postmortem examination revealed no evidence of any significant natural disease process, physical violence, or trauma. The stomach contained 275cc of green leafy material with a small amount of dark red to brown admixed liquid. The proximal duodenum contained a small amount of similar green leafy material. No pills or capsules were identified in the stomach. Confirmatory testing is pending at this time, but circumstantial evidence indicates that the decedent died from intentional hemlock ingestion, and the death certificate has been finalized.

**Discussion:** Ingestion of hemlock is a rare method of suicide. Botany examination of the leafy material recovered from the decedent's stomach contents confirmed that the material was consistent with *Conium maculatum*, also known as poison hemlock. This case emphasizes the need for further investigation regarding hemlock intoxication, as diagnosis of hemlock poisoning is challenging and there is a lack of scholarship on its biological half-life, levels at which hemlock is fatal, and human metabolism of hemlock alkaloids.

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#### Hemlock, Intoxication, Suicide



### H32 A Fatality by Caustic Soda: Accidental or Suicidal?

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**Learning Overview:** After attending this presentation, attendees will understand the pattern of injuries caused by Caustic Soda (NaOH) on the skin surface, the mucosa of the mouth, and internally. Attendees will understand the classification of esophageal and gastric caustic in addition to postmortem findings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving to identify and ascertain cause and manner of death in a fatality by NaOH ingestion. This presentation will also be helpful in providing education regarding current guidelines in the management of esophageal caustic injury.

NaOH is a strong base with a very high corrosive potential. In Indian households, NaOH is used for making soap by an empirical method in many poorer regions. Caustic ingestion represents a public health problem worldwide, despite efforts and educational campaigns to reduce its occurrence. Ingestion is the most frequent route of exposure. Accidental ingestion most typically occurs in infants and children less than six years of age, especially between the ages of 1–4 years. Otherwise, ingestion in adolescents and adults may be related to suicidal behaviors. In the United States, an incidence of 5,000 to 15,000 cases per year are estimated to occur. The type of corrosive agents varies from country to country. Reports from Denmark, Israel, the United Kingdom, Spain, and Australia showed that although alkaline agents are most commonly used in developing countries, the majority of caustic ingestion is due to acids. Caustic ingestion induces a local toxicity leading to upper gastrointestinal system damage that can range from mild to extensive injuries.

The current case is that of a 76-year-old woman who presented with a medical history significant for a cataract in one eye and suspected consumption of NaOH. Corrosive injury was present over the lips and tongue. Esophageal endoscopic examination revealed grade IIb injury. Circumferential lesions with ulceration focally extending into the muscle layers was apparent. The stomach revealed grade IIIb injury; the gastric mucosa appeared dusky, very black, with deep ulcerations (transmural tissue involvement was suspected). The patient died and an autopsy was requested. On postmortem examination corrosive injury was confirmed to extend over lips and tongue and appeared brownish in color. On internal examination, brown-colored corrosive injury extended over the esophagus with resultant narrowing of the lumen. The stomach demonstrated corrosive injury involving the pylorus that likewise appeared brown in color. Further detailed history and crime scene investigation revealed that a bottle of NaOH was found near the woman. The investigating officer queried the manner of death. Further case history will be reviewed as part of this presentation.

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**Caustic Soda, Accidental, Suicidal**

### H33 The Evolution of Safety Systems in Traumatic Deaths Due to Road Traffic Accidents: A Case Report and Review of the Literature

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**Learning Overview:** After attending this presentation, attendees will be more aware of safety systems in traumatic deaths due to road traffic accidents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by underlining the role of new safety devices in the reduction of fatal events in impacts between motor vehicles.

In 2016, there were 175,791 road traffic accidents in Italy resulting in 3,283 deaths (i.e., dead within 30 days of the accident) and 249,175 injured victims.<sup>1</sup> According to the Italian National Institute of Statistics (ISTAT) data, the number of road traffic accidents overall has decreased. This decrease is likely attributable to new legislation on the reduction of the driving license score (i.e., a law introduced on July 1, 2003, in Italy), as well as the increased active and passive safety systems newer vehicles are equipped with. The role of seat belts has also been established, though more focus in scientific studies has recently been given to other security systems, such as airbags. The causes of road accidents are many, but major factors appear to be distraction, imprudence, and high speed. In a significant percentage of cases—particularly those involving young people—driving while under the influence of alcohol plays a major role.<sup>2,3</sup> The injuries and consequences that occur as a result of road traffic accidents are diverse and depend on many variables. These may include bruises, fractures of the limbs, brain injuries, visceral ruptures, pulpification, cervical spinal injury (more frequently seen in motorcycle accidents), or other serious consequences leading to immediate death. The introduction of the new technology in road vehicle safety systems has allowed for a reduction in adverse outcomes secondary to road accidents, although some studies have noted that the use of seat belts can actually result in neck ecchymoses, carotid thrombosis, fracture of the aortic arch, clavicular or costal fractures, pneumothorax, and abdominopelvic lesions due to strong traction during an impact.

This presentation reports the case of an elderly man found dead inside an Ape Classic<sup>®</sup> vehicle due to a frontal motor vehicle collision. Scene investigation revealed that the other vehicle involved in the collision was a common modern car. Injuries to the elderly man, including an abdomen extensively lacerated by the mechanical components of the handlebar of the Ape Classic<sup>®</sup> that precluded easy removal of the body. The kinematic engineering consultancy showed that the Ape Classic<sup>®</sup> vehicle was traveling at a low speed. An autopsy was performed. External examination showed an abdominal wound about 18–20cm deep with jagged margins and an internal foreign body belonging to the handlebar of the Ape Classic<sup>®</sup>. In the lower limbs, there was a contused laceration of the knee with tibial and femoral fracture. On internal examination, there was a splenic laceration, massive traumatic hemoperitoneum, rupture of the rectum and inferior mesenteric intestinal loops with right-sided pelvic/sacral traumatic fractures due to the foreign body. Toxicological analysis was negative for alcohol and drugs in both the victim as well as the driver of the other car. A histopathologic assessment was performed. All collected data was reviewed in order to better understand the causes and the dynamics of the accident. Overall, it appeared the decisive element in the different evolution of the outcomes of the described incident was the intrinsic characteristics of the vehicles involved. The Ape Classic<sup>®</sup> was totally devoid of the common active and passive safety systems that were present in the other vehicle involved in the collision. As a result, the elderly man was mortally wounded by the impact; whereas, the driver of the other vehicle lived.

The frontal motor vehicle collision reported in this case confirms that the presence of airbags or the evolution of vehicle safety systems would have almost certainly prevented the deep abdominal wound caused by the impact on the steering wheel and the windshield, and ultimately the victim's death. In conclusion, the role safety belts play in the reduction of fatal events in the impacts between motor vehicles is emphasized. It is also noted that although most newer vehicles have safety systems, many of those that are often involved in road accidents are older and thus may have limited-to-no safety devices. An evolution of road regulations is needed in order to reduce fatal events due to the lack of vehicle safety systems.

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**Forensic Science, Road Traffic Accident, Autopsy**





### H34 Is the Survey of Forensic Botany Useful? The Application and Limits of Forensic Case Analysis

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**Learning Overview:** After attending this presentation, attendees will understand the role of forensic botany in crime scene investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the influence of forensic botany in the evaluation of manner of death, in analyzing the dynamics of death, and in clarifying the primary crime scene.

In recent years, botany has become an extremely important tool for forensic analysis. The study of plants and their alterations as a result of the dynamics of criminal events is able to provide critical interpretative information during the judicial inspection of the crime scene and may inform the determination of manner of death. In this study, each reported case involved a detailed scene analysis performed at least twice. In each judicial inspection, all the botanical elements were analyzed and cataloged. In addition, botanical analyses were carried out on the clothing and/or on the victims' bodies. In cases in which multiple botanical elements were found, a method of taxonomic comparison was applied between the environmental elements with the vegetative elements found on the corpse. Each element was analyzed macroscopically and compared with photographic surveys. This presentation reports six forensic cases in which forensic botany analysis was employed. The purpose of this study is to better understand and characterize the nature of the forensic cases in which botanic studies prove most useful.

In the first case, the suspected suicide of a girl was analyzed, due to a fall from a height. The dynamics of the fall were not initially clear. On the girl's hair and clothing, investigators found botanical elements that were compared to plant elements of a garden hedge. This analysis allowed for the reconstruction of the impact point and the subsequent projection during the fall, thus clarifying the mode of death.

The second case involved a man's sharp force slash murder. During the inspection and at the autopsy, botanical elements were found on the neck in the region of the sharp force injuries, together with other elements on the back that were completely crushed and mixed with soil residues overlying a large lumbosacral ecchymosis. These botanical data, together with the autopsy evidence, suggested that the victim was dragged after the murder and clarified the position of the body at a different point from the site of the murder.

The third case concerns a boy found dead due to a suspected pedestrian versus motor vehicle collision, albeit at a considerable distance from the accident site. Neither the dynamics of the collision nor the driver's statements regarding the impact were clear. The presence of botanical elements on the corpse suggested the actual point of impact of the corpse following the projection due to the impact with the car.

The fourth case concerns a demented elderly woman who was found dead following a mid-height fall. At the autopsy, there were small botanical elements that suggested the path taken by the woman before the fall, also clarifying the mode of death.

The fifth case concerns a boy's fall from a height. The discovery of specific botanical elements overlying the soles of the shoes and on clothing suggested the boy's fall from an area made up of bushes at a height of about 20 meters.

The sixth case concerns the death of a man with electrical and other traumatic injuries who was found in an apartment. The analysis of botanical elements in the hair directed the investigators to search for another crime scene, clarifying the dynamics of death that occurred accidentally during the theft of copper from high voltage pylons.

In total, these cases show that forensic botany can be an important aid in investigations for: (1) clarifying the method of death; (2) analyzing the points of loss of balance and impact in falls; (3) clarifying the dynamics of suicides or accidental traumatic events, such as pedestrian versus motor vehicle accidents; (4) identifying the primary crime scene with respect to a secondary one; (5) unveiling false crime scenes; and (6) revealing the dynamics of concealment of the corpse in cases of homicide or displacement of the body.

Certainly, this investigation is limited by the wide geographical areas, by the state of integrity of the analyzed elements, and by the timing with which the investigations are carried out. To date, the standard analytical method is the macroscopic and microscopic comparison of the elements in their taxonomic characteristics.

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#### Forensic Sciences, Forensic Botany, Autopsy

### H35 The Immunohistochemical Analysis in the Diagnosis of Freshwater Versus Saltwater Drowning: A Case Report and a Review of the Literature

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**Learning Overview:** After attending this presentation, attendees will be able to describe which investigations are most useful in cases of death due to drowning.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of immunohistochemical analysis and the need for the research of a pathognomonic parameter for the differential diagnosis between freshwater and saltwater drowning.

Drowning is one of the most frequent causes of death. According to recent epidemiologic research, the incidence is about 400,000 deaths per year in the world, of which 30,000 occur in Europe.<sup>1</sup> In most cases, these are accidental deaths or suicides.<sup>2</sup> Drowning is a form of asphyxia. The liquid filling the airways replaces air contents resulting in pulmonary edema and anoxic-ischemic encephalopathy. The pathophysiology of drowning depends on the characteristics of the inhaled liquid. If it is hypertonic (i.e., saltwater), the consequences will be hemoconcentration with hypovolemia, sodium, calcium, and magnesium increase, impaired cardiac function (without ventricular fibrillation), and massive pulmonary edema. Death occurs in 8–9 minutes. If the liquid is hypotonic (i.e., freshwater), it will inactivate the surfactant and is rapidly absorbed into the bloodstream, inducing hypervolemia, dilution anemia, and hemolysis with the release of intracellular potassium and lethal ventricular fibrillation. Death occurs in 3–5 minutes. Foaming at the mouth, cyanosis, bright red color hypostasis, and goosebumps are some indicative external signs. Histologic examination plays an essential role. It can establish the presence of irregular areas of dilatation and rupture of alveolar trabeculae, hemorrhage, and edema. The purpose of this work is to compare forensic investigation results in freshwater versus saltwater drowning.

Two forensic cases are presented. The first case is that of a man who fell from a boat into the Ionian Sea. The second case is that of a man who fell into the water of a lake. Autopsy, cartometric testing, and histopathologic and toxicologic examination were performed in both cases. In both cases, external examination showed signs indicative of drowning. In the first case, the lungs were emphysematous and crackling with evidence of petechiae and pulmonary edema at autopsy. Cartometric testing showed greater hemodilution of blood within the right ventricle as compared to the left. In the second case, the lungs appeared congested and edematous. Cartometric testing showed greater hemodilution of the blood within the left ventricle as compared to the right. The renal and cerebral immunohistochemical analyses showed a different expression of aquaporins in the two presented cases. The results obtained from these investigations were compared with those found in a non-systematic review of the literature conducted via the PubMed NCBI search engine.

The diagnosis of drowning remains a challenge for the forensic pathologist. There is no single pathognomonic autopsy finding, especially with regard to distinguishing between freshwater and saltwater drowning. As presented in these cases, the cartometric test and, above all, the immunohistochemical analysis are fundamental in the forensic investigation. The cartometric test entails dropping blood taken from the ventricles onto a sheet of bibula paper and subsequently observing the size and color tone of the marks. In cases of hemodilution, the blood from the left ventricle will leave a larger and lighter red-colored mark than the right (e.g., as in the second presented case). The opposite occurs in cases of drowning in saltwater (e.g., as in the first presented case). Immunohistochemical investigations already play an important role in distinguishing between drowning and other types of asphyxia.<sup>3,4</sup> In differentiating between freshwater and saltwater drowning, it is important to evaluate the expression of aquaporins, a protein-channel family responsible for transmembrane passage of liquids. Their expression at tissue level varies according to changes in plasma osmolarity. In particular, in cases of freshwater drowning, aquaporin-2—which is normally found on the renal collecting ducts—is hypo-expressed, while the aquaporin-4—normally found in astrocytes—is hyper-expressed.<sup>5,6</sup> The opposite pattern is seen in cases of saltwater drowning. The two cases presented here confirm these expression patterns. Literature highlights how the study of aquaporin-5—normally expressed in type I pneumocytes—is more useful to distinguish between drowning in fresh water and postmortem immersion.<sup>7</sup> At present, the investigations described herein are the most reliable and accurate means of distinguishing fresh versus saltwater drowning, as there is not yet a single pathognomonic histopathologic marker.

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#### Forensic Sciences, Drowning, Immunohistochemical Analysis



### H36 Death From Pheochromocytoma Initially Presenting as a Suspected Homicide

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**Learning Overview:** After attending this presentation, attendees will be aware of the importance of performing autopsies in all cases of suspected homicide. Also, this case will provide an additional example to the forensic literature of a natural death that initially presented as a suspected homicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that a natural death, in this case, one related to a pheochromocytoma in conjunction with postmortem animal activity and suspicious scene findings, can mimic homicidal violence.

**Introduction:** One of the most important aspects of medicolegal death investigations involves the examination and documentation of homicidal deaths. Appropriate evaluation of such cases, including evidence collection, may have far-reaching implications for subsequent adjudication. In this report, the case of a suspicious death which, after complete investigation and autopsy, was determined to be caused by a pheochromocytoma is presented.

**Case Report:** A 66-year-old man was found unresponsive by Emergency Medical Services (EMS) at his residence in an isolated pole barn. After confirming the man was deceased, the on-scene police officers immediately labeled the death suspicious, as initial signs indicated violence had occurred, with evidence of a significant injury on the decedent's head and multiple guns and power saws at the scene. Additionally, skin and flesh were noted to be missing from the decedent's left ring finger and middle finger. Mice were noted to be present within the barn. Medical history, provided by the decedent's friend who called 911, revealed that the patient had complained of flu-like symptoms in the week prior to his death. Upon further investigation, most of the head injuries appeared to be consistent with postmortem rodent feeding, with no lethal injuries identified. Autopsy revealed a 7cm, mostly solid mass in the right adrenal medulla, as well as a markedly enlarged heart (750g), with coronary artery atherosclerosis, and kidney findings consistent with hypertension. Adrenal tumor cells stained positive for chromogranin A, synaptophysin, and neuron-specific enolase, confirming the diagnosis of pheochromocytoma. The cause of death was hypertensive and atherosclerotic cardiovascular disease, with a contributing factor of an underlying pheochromocytoma. The manner of death was natural.

**Discussion:** The full investigation and autopsy in this case highlight the importance of considering rare diseases as potential causes of death. While adrenal pheochromocytoma may be classified as a rare disease, nearly 50% of pheochromocytomas are first diagnosed at the time of autopsy.<sup>1</sup> The other half tend to be discovered incidentally. Pheochromocytomas are tumors that arise from the chromaffin cells of the adrenal medulla. While they are usually benign, these tumors can often secrete excess levels of catecholamines, such as epinephrine and dopamine, causing a wide range of adverse effects. Diagnosis is complicated by the wide range of non-specific, sometimes hidden, clinical symptoms. One of the hallmark symptoms of a pheochromocytoma is hypertension. The clinical triad widely used to suspect these tumors consists of headache, palpitation, and diaphoresis. Pheochromocytomas may also present with anxiety, chest pain, dyspnea, nausea, and vomiting. Such symptoms are common in a wide range of diseases and tend to broaden the differential. From a forensic perspective, this case represents an example of a natural death that initially presented as a homicide. While both accidental traumatic and natural deaths initially presenting as homicides have been reported in the forensic literature, such cases are uncommon and often puzzling. Only via cooperative efforts between law enforcement and death investigators, along with a detailed postmortem examination, can homicide be conclusively ruled out and the true nature of the death be recognized.

**Conclusion:** This case initially presented as a suspected homicide due to traumatic postmortem animal activity; however, the true cause of death was related to an underlying pheochromocytoma. This case serves to educate forensic professionals and law enforcement officials that natural deaths can occasionally be mistaken as homicides. This case also serves to raise awareness in the medical community of the variable clinical presentation of pheochromocytomas as well as the disease's sometimes silent nature.

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#### Pheochromocytoma, Sudden Death, Suspected Homicide

### H37 A Spontaneous Aortic Rupture: A Report of Two Cases

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**Learning Overview:** After attending this presentation, attendees will understand the concept of spontaneous aortic rupture. Through a detailed pathological analysis in addition to a ten-year review of the literature, this study sought to clarify the clinico-pathologic features of this uncommon entity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a postmortem diagnosis in the sudden cardiac death and treatment of a spontaneous aortic rupture.

**Background:** Acute aortic syndromes comprise a heterogeneous group of life-threatening disorders and are characterized by acute-onset chest or back pain with or without cardiovascular collapse.<sup>1</sup> The major syndrome entities include acute aortic dissection, penetrating atherosclerotic ulcer, and intramural hematoma.<sup>2</sup> Spontaneous rupture of the ascending aorta in the absence of trauma and without antecedent aneurysm or dissection is one of the rare causes of sudden cardiac death.<sup>3</sup>

**Case Reports:** Two female decedents are presented with similar aortic pathologic findings. One was 68 years old and had been admitted to a private health care facility with uremic encephalopathy arising in a background of chronic kidney disease and hypertension. This was followed by sudden onset of breathlessness and altered sensorium. The second woman was 70 years old with hypertension. She presented with retrosternal chest pain and breathlessness, followed by unconsciousness.

**Material and Methods:** Complete autopsies were performed in both cases.

**Results:** Both hearts showed hemopericardium that developed as a result of a circumferential through-and-through tear of the proximal ascending aortic wall (unlike acute aortic dissection) with adventitial rupture. A circumferential tear, seen in relation to uncomplicated atherosclerotic plaques, was present 4.2cm above the sinu-tubular junction and 0.5cm proximal to the origin of the right brachio-cephalic artery, respectively. Hemopericardium and resultant cardiac tamponade occurred in the two elderly hypertensive women due to almost identical pathology. In each case, there was a tear, through and through, involving both the intima and the media with no or limited dissection of the ascending aorta. Spontaneous Aortic Rupture (SAR) is defined, in its broadest sense, as a non-traumatic simultaneous rupture of the intima and media of a non-aneurysmal aorta, when other acute aortic syndrome pathologies have been ruled out by appropriate imaging modalities and/or histopathological studies of the surgically excised segments or at autopsy.<sup>4</sup> Thus, both cases were designated as SARs.

**Conclusion:** When acute intrapericardial bleeding develops with no evidence of aortic aneurysm or dissection, spontaneous aortic rupture should be suspected. It is important to keep SAR in the differential diagnosis of patients presenting with chest pain and cardiovascular collapse so that timely and emergency intervention can be deployed to ensure better chances of survival. But forensic pathologists should be aware that rapid onset, misdiagnosis, and unpredictability may result in rapid disease progression and death; thus, diagnosis may not be made until the time of postmortem examination. Since the diagnosis may elude even the most experienced and knowledgeable of clinicians, missing the diagnosis does not necessarily represent negligent medical care. At the same time, the clinical findings, differential diagnosis, investigations, treatment plan, and follow-up instructions should be documented scrupulously.

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#### Spontaneous Aortic Rupture, Acute Aortic Syndrome, Sudden Cardiac Death



### H38 HIV Post-Sudden Cardiac Death (SCD): Rates of Autopsy-Defined Sudden Arrhythmic Death (SAD) Are 80% Higher in Persons With HIV

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**Learning Overview:** This presentation will show that autopsy material informs the living. After attending this presentation, attendees will know that certain chronic HIV infection increases the risk of sudden death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that HIV-infected persons are more likely to die suddenly of an arrhythmic death, and they may benefit from the implantation of a defibrillator.

**Background:** Persons living with HIV have higher rates of Cardiovascular Disease (CVD), including acute myocardial infarct and heart failure. High rates of out-of-hospital presumed SCDs were first reported using World Health Organization (WHO) criteria.<sup>1</sup> However, the precise incidence of actual SADs in HIV remains unknown.

**Methods:** Between 2011 to 2016, this study prospectively identified all incident deaths attributable to out-of-hospital cardiac arrest among individuals with and without HIV aged 18–90 years in San Francisco County for medical records review and comprehensive autopsy, toxicology, and histology via medical examiner surveillance of consecutive out-of-hospital deaths. Autopsy-defined SAD had no extracardiac cause of death or acute heart failure. Final cause was adjudicated by a committee of pathologists, cardiologists, HIV clinicians, and electrophysiologists.

**Results:** One hundred twenty-six out-of-hospital HIV-infected deaths were identified, and 47 of these met WHO SCD criteria. The mean age was 65.6 years, 94% were male, 57% were White. Compared to uninfected WHO-defined (presumed) SCDs ( $N=505$ ), SCDs with HIV were more likely to have a history of myocardial infarct, psychiatric disorder, cigarette smoking, and substance abuse. About half (22 of 47, 47%) of WHO-defined SCDs were autopsy-defined SADs; the remainder were non-cardiac and included 16 due to occult overdose. Presumed SCDs with HIV were more likely to be due to occult overdose (13% vs. 34%,  $p<0.0001$ ) and renal failure (1% vs. 6%,  $p=0.0031$ ) as compared to uninfected presumed SCDs. Adjusted incidence ratios for WHO (presumed) SCDs and autopsy defined SADs were both significantly higher in HIV (IRR 1.82, 95%CI 1.4–2.4,  $p<0.0005$  and IRR 1.83, 95%CI 1.2–2.8,  $P=0.006$ , respectively). After adjustment for age, gender, heart disease, and Coronary Artery Disease (CAD), SCDs with HIV had 60% higher interstitial fibrosis by myocardial trichrome staining compared to uninfected SCDs.

**Conclusions:** In this countywide postmortem study, one-third of apparent SCDs in HIV over a five-year period were due to occult overdose. However, adjusted rates of both presumed SCDs and autopsy-defined SADs were 82% and 83% higher, respectively, in HIV compared to the uninfected population. Higher levels of cardiac fibrosis in HIV, a known substrate for SAD in the general population, may underlie the mechanism by which HIV increases risk for SAD. Development of criteria and evaluation for implantable defibrillators in HIV should be carefully considered in the future as a means to prevent SAD in this high risk population. The underlying cause of increased cardiac fibrosis in HIV-infected persons is under investigation by this study group and will be the subject of a future publication.

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#### Sudden Death, HIV, Autopsy



### H39 A Fatal Case of Histoplasmosis With Colonic Perforation in a Patient With Acquired Immune Deficiency Syndrome (AIDS)

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**Learning Overview:** The goal of this presentation is to introduce a case of extrapulmonary histoplasmosis in a patient with a postmortem diagnosis of AIDS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing an interesting presentation of an AIDS-defining illness postmortem. This presentation will also address the utility of obtaining serum at autopsy for postmortem testing.

*Histoplasma capsulatum* is a fungal organism most prevalent in the Ohio and Mississippi River Valleys. Humans are exposed to *Histoplasma* through the environment, especially soil contaminated with bird or bat droppings. Typical infections are pulmonary and self-limited, although immunocompromised patients are at risk for severe and potentially fatal infections. Histoplasmosis, extrapulmonary or disseminated, is an AIDS-defining illness.

The goal of this case presentation is to introduce a fatal case of disseminated histoplasmosis in a patient with undiagnosed AIDS. A 41-year-old male inmate with a past medical history of diabetes mellitus and hypertension was found unresponsive in his cell. Death was pronounced soon after discovery, and an autopsy was ordered. There was no evidence of foul play at the scene and the decedent had no significant mental health history. The autopsy was performed at the West Tennessee Regional Forensic Center in Memphis, TN.

External examination was unremarkable with no evidence of trauma. Internal examination revealed a fibrinous peritonitis associated with 350 milliliters of turbid ascites fluid. A colonic perforation was identified, distal to the splenic flexure, with extensive induration of the adjacent mesentery and retroperitoneal tissue. Additional findings at autopsy consisted of cardiomegaly and coronary artery atherosclerosis. Histologic examination of the colonic perforation site showed granulomatous inflammation with granulation tissue. Histologic examination of the liver revealed small periportal and intralobular granulomas with periportal-based mononuclear cell infiltrates. A Gomori Methamine Silver (GMS) stain was ordered and highlighted intracellular and extracellular organisms morphologically consistent with *Histoplasma* within the colon and liver sections.

The West Tennessee Regional Forensic Center in Memphis, N, attempts to collect blood from all decedents in a serum separator tube in the event postmortem serum testing is warranted. Blood may also be collected in Ethylenediaminetetraacetic Acid (EDTA) -containing tubes in select cases in the event molecular testing is indicated. Due to the extrapulmonary and disseminated nature of the decedent's infection combined with the lack of known predisposing risk factors, Human Immunodeficiency Virus (HIV) combined antibody and antigen testing on postmortem serum was ordered. The result of the combined testing was positive and identified to be HIV-1 by confirmatory antibody testing. The cause of death in this case was determined to be peritonitis secondary to colonic histoplasmosis with perforation. Given the positive postmortem HIV testing and that extrapulmonary histoplasmosis is an AIDS-defining illness, the underlying cause of death was certified as AIDS.

This case exhibits the importance of obtaining postmortem serum in the event additional laboratory testing is warranted secondary to findings at autopsy. The presence of extrapulmonary disease prompted postmortem testing to determine the HIV status in this case, also highlighting the importance of knowledge regarding AIDS-defining illnesses in postmortem evaluations.

#### **Histoplasmosis, Human Immunodeficiency Virus, Colonic Perforation**



### H40 The Implementation of a Forensic Pathology Rotation for Medical Students

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**Learning Overview:** After attending this presentation, attendees will be exposed to the inception, implementation, execution, and management of the El Paso County Office of the Medical Examiner (EPOME) elective forensic pathology rotation for medical students from the Texas Tech Paul L. Foster School of Medicine (PLFSOM). This experience provides an early introduction to forensic pathology to medical students through an educational curriculum that has performance objectives carefully aligned with the Accreditation Council for Graduate Medical Education (ACGME) core competencies for Graduate Medical Education (GME).

**Impact on the Forensic Science Community:** The forensic science community will be impacted by this presentation in that it provides an effective blueprint to managing, implementing, and evaluating the project of a student rotation in a forensic field. Promoting early visibility of forensic sciences through high-quality, meaningful exposure has been mentioned as one of the key strategies that can contribute to individuals choosing forensic specialization fields. The rotation outlined in this presentation has accomplished that by: (1) allowing students to acquire basic knowledge of forensic pathology; (2) teaching students advanced forensic autopsy techniques; (3) allowing students to become familiar with the daily duties of a forensic pathologist, such as daily interactions (with family, attorneys, other medical professionals), courtroom testimony, administrative issues; (4) providing students with literature and resources to broaden their fund of knowledge; (5) mentoring students on academic projects; and (6) supporting the students' applications to pathology residency positions.

Prior to this rotation, the autopsy pathology exposure the medical students from the PLFSOM experienced was limited to a 4-week Laboratory Medicine rotation, which included time spent in both the anatomic and clinical pathology labs with limited, if any, hospital autopsy exposure. Following implementation of an elective forensic pathology rotation, the students have had the opportunity to view daily autopsy cases in a medical examiner setting, participate in morning conferences where cases are presented, attend lectures provided by the faculty, join slide review sessions, and have graduated responsibilities in the autopsy suite, up to taking ownership of forensic autopsy cases, including formulating opinions on ancillary testing (histology, toxicology) and learning how to produce formal, written autopsy reports.

This presentation is intended to outline key project management steps, including: defining the project, setting goals and milestones, putting together a team, monitoring tasks, keeping stakeholders informed and increasing their buy-in, and bringing the project to completion. Also described are monitoring strategies to ensure continued quality improvement as well as ongoing strategies to optimize the didactic value of the student experience (e.g., student customization of the rotation, bidirectional feedback, and aligning the academic sessions with the students' ongoing curriculum).

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#### Medical Education, Student Rotation, Forensic Pathology



### H41 A Molecular Diagnostic Determination of Human vs. Bovine Origin of Viral-Associated Aggressive Natural Killer (NK) Cell Leukemia

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**Learning Overview:** After attending this presentation, attendees will have: (1) gained familiarity with diagnostic features of aggressive NK cell leukemia, a fulminant malignancy that may remain undetected until the time of postmortem examination; (2) developed an understanding of the critical role viruses can play in the development of such malignancies; and (3) appreciation for the utility of molecular diagnostic testing in elucidating the nature/source of viral infections.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of collaborating with pathology specialties, such as molecular and hematopathology, in order to more effectively investigate and accurately certify deaths in cases of suspected viral-associated hematologic malignancies.

A 51-year-old Hispanic male with no known medical history who worked on a dairy farm presented with acute hepatic failure of unknown origin three days prior to his death. Laboratory testing in process at the time of death ultimately revealed quantitative Epstein-Barr Virus (EBV; *human gammaherpesvirus 4*) DNA copies at >1,000,000IU/mL. At autopsy, external and internal examination was notable for diffuse jaundice, anasarca, and evidence of disseminated intravascular coagulation. The liver revealed a background of micronodular cirrhosis with superimposed acute necrosis. There was marked splenomegaly (1,290g) with extensive geographic necrosis. Hepatosplenic histologic sections showed atypical lymphocytes that were positive for EBV-encoded RNA (EBER) and CD56, consistent with EBV-infected NK cells. Circulating EBV-infected NK cells were also seen in sections of vessels. Overall these findings—in conjunction with the patient's fulminant clinical presentation—appeared consistent with EBV-associated aggressive NK cell leukemia.

The state Occupational Safety and Health Administration (OSHA) was subsequently contacted due to a third parties' concern that this represented a work place-acquired viral infection and death. While there is a *bovine gammaherpesvirus 4* (BHV-4) linked to respiratory and reproductive clinical manifestations in dairy cattle, it is not known to commonly infect and/or cause disease in humans. Due to presumed genetic similarity between the human (EBV) and bovine (BHV-4) herpesviruses, there was concern that the Polymerase Chain Reaction (PCR) primer used for the antemortem EBV test could have amplified BHV-4, and thus been erroneously reported as an EBV DNA PCR copy number. To address this concern, real-time PCR (rtPCR) was repeated on a patient blood sample obtained at the time of autopsy, and the amplicon generated from rtPCR was Sanger sequenced. Sequence comparison of the amplification product to various nucleotide sequence databases using Basic Local Alignment Search Tool confirmed the viral infection to be EBV (human origin) rather than BHV-4 (bovine origin).

EBV is one of the most common human viruses in the world. Antibodies to EBV have been demonstrated in all population groups with a worldwide distribution; approximately 90%–95% of adults are EBV-seropositive. EBV infection has been associated with the development of an array of hematologic malignancies, including NK cell leukemia. Aggressive NK cell leukemia is a rare hematologic malignancy that is most commonly seen in Asia, Central America, and South America. The disease has a highly aggressive course with a median survival of <2 months. High levels of circulating plasma EBV at the time of diagnosis (as were found in the current case) have been suggested to portend a worse prognosis. Hepatosplenomegaly and acute hepatic failure have been reported in conjunction with aggressive NK cell leukemia. In conclusion, the cause of death in this case was fulminant acute hepatic failure due to EBV-associated aggressive NK cell leukemia. This case serves to illustrate the utility of molecular diagnostic testing in distinguishing human vs. bovine viral origin in cases in which there is concern for occupational exposure to the infectious agent.

#### Molecular Diagnostics, EBV, Aggressive NK Cell Leukemia





### H42 Neurodegeneration in the Forensic Setting: General Principals and Diagnostic Applications

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**Learning Overview:** After attending this presentation, attendees will learn how to diagnose Alzheimer Disease (AD), Lewy Body Disease (LBD), and Frontotemporal Degeneration (FTD) by performing a cost-effective dementia workup on decedents who were reported to be cognitively impaired before death and will see several examples of these practical workups.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demystifying the diagnostic criteria for the most common neurodegenerative diseases and by providing a way to reasonably diagnose these conditions with minimal tissue sampling and immunohistochemical workup.

The incidence of neurodegenerative conditions is continuing to increase as the population ages. The most common of these disorders is AD, followed by LBD and FTD. The prevalence of these conditions is projected to exponentially increase over the next several decades and, without effective treatments, the costs to society will be catastrophic. Neurodegenerative disorders frequently contribute to non-natural deaths in the elderly by increasing the likelihood of devastating falls, choking, and wandering activity, which can lead to lethal environmental exposures and other injuries. As such, an ever-increasing number of elderly deaths are falling under medical examiner/coroner jurisdiction. These degenerative disorders can only be definitely diagnosed at autopsy and many forensic pathologists find these assessments difficult. Additionally, the postmortem workup of these disorders can be expensive, and many offices cannot afford to routinely perform the comprehensive assessment recommended by the National Institute of Aging-Alzheimer's Association (NIA-AA) guidelines.

To help combat this problem and empower forensic pathologists to more confidently perform their own assessments, this study proposes a condensed workup that includes sampling the hippocampus, frontal lobe, occipital lobe, basal ganglia, and midbrain that can be performed using 2–3 tissue cassettes. Immunohistochemical staining of these blocks with  $\beta$ -amyloid, tau, and  $\alpha$ -synuclein should be sufficient to accurately diagnose most causes of neurodegeneration in the cognitively impaired elderly. Even without these immunostains, a great deal of information can still be obtained by looking for neuritic plaques, neurofibrillary tangles, and Lewy bodies with routine hematoxylin and eosin staining. Herein, several autopsy cases of individuals with cognitive impairment are reviewed utilizing this postmortem protocol with final diagnoses, including AD, LBD, and FTD. The accurate pathologic diagnosis of dementing disorders can have a significant impact on a decedent's family and help resolve certain medicolegal issues surrounding the death. Additionally, more accurate data about the prevalence of these diseases is required to understand their contribution to deaths, especially in the forensic setting.

#### Neurodegeneration, Dementia, Alzheimer Disease



### H43 Hypothermia-Related Deaths: A Ten-Year Retrospective Study of Two Major Metropolitan Cities in the United States

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**Learning Overview:** After attending this presentation, attendees will understand the epidemiology, risk factors, autopsy, and toxicological findings associated with hypothermia-related deaths during the past ten years in New York City, NY, and Houston, TX.

**Impact on the Forensic Science Community:** This presentation will impact the forensic and public health communities by identifying the risk factors for these fatalities in large metropolitan areas and will assist in reducing these deaths in susceptible populations.

Deaths due to hypothermia are preventable and affect vulnerable populations, including the homeless, elderly, and people with substance abuse and mental illness. Hypothermia is defined as a core body temperature of less than 95°F.<sup>1</sup> There are approximately 1,300 deaths due to hypothermia in the United States every year, most of which occur in the Midwest or West; however, Southern states may exhibit rapid temperature drops at night, in stark contrast to the daytime heat, leaving people unprepared.<sup>2-4</sup> The postmortem diagnosis of hypothermia can be challenging, as there are no pathognomonic signs.<sup>5</sup> This study reviewed the medical examiner case files on all deaths due to hypothermia over a ten-year period in two large populous cities in distinct geographic regions of the United States (New York City and Houston) to study the autopsy findings, toxicologic results, and the epidemiologic patterns of these fatalities.

The New York City Office of Chief Medical Examiner and Harris County Institute of Forensic Sciences electronic databases were searched for all fatalities where the cause of death included hypothermia, between January 2009 and July 2019. There were a total of 189 fatalities (139 in New York City, and 50 in Houston). The following data was collected: age, sex, ethnicity, survival interval, manner of death, place of death, place found, initial body temperature, outdoor temperature (high and low), precipitation, residence, autopsy findings, and toxicological results.

Of the 189 fatalities, there were 143 males and 46 females, and the average age was 60.1 years in New York and 64 years in Houston; age ranged from a neonate to 97 years old. The ethnicities were Black ( $n=76$ ), White ( $n=61$ ), Hispanic ( $n=39$ ), and Asian ( $n=13$ ). The known number of homeless fatalities ( $n=84$ ) versus those with a fixed residence ( $n=93$ ) was similar. A survival interval occurred in 91 cases, ranging from the time required to complete the rewarming protocol (hours) up to 33 days, of whom, the coldest recorded body temperature was 68.3°F, and the highest was 92°F. The average daytime high temperature in New York was 41.7°F and in Houston was 54.4°F; the average nighttime low in New York was 27.2°F and in Houston was 36.1°F. Precipitation (rain and snow) was noted in the weather report for 56 cases. The most cases by year for both cities was 2018, with a total of 23 in New York and 11 in Houston.

All causes of death were due to hypothermia and the manner of death was divided among accident ( $n=181$ ), undetermined ( $n=5$ ) and suicide ( $n=3$ ). The most common autopsy findings were Wischnewski spots in the stomach ( $n=107$ ) and pancreatic hemorrhage ( $n=29$ ). Toxicologic analysis detected ethanol ( $n=69$ ) with an average blood concentration of 0.20gm% in New York, and 0.27gm% in Houston; cocaine ( $n=9$ ); and others, including opioids, benzodiazepines, stimulants, and antipsychotics were more rarely detected. Other postmortem findings included cardiovascular disease ( $n=81$ ) and chronic obstructive pulmonary disease ( $n=16$ ). Pre-existing diagnoses of dementia ( $n=8$ ) or a mental illness, such as schizophrenia or bipolar disorder, ( $n=14$ ) were also noted.

Hypothermia is an important cause of preventable weather-related deaths in major metropolitan cities in the United States. Despite the different geographic locations and climates of New York City and Houston, many similarities were noted between the patient populations, including male sex, advancing age, and the number of deaths occurring equally between homeless people and those with a fixed residence, suggesting that even people who have access to shelter are at risk. Local agencies can use this data to target these higher-risk populations and offer appropriate intervention to try to prevent these deaths. The lack of pathognomonic autopsy findings emphasizes the importance of a thorough scene investigation; otherwise, these deaths may be underreported. The role of the medical examiner is crucial in the accurate classification of these deaths, to assist in improving public education, targeted interventions, and emergency response planning.

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#### Hypothermia, Weather-Related Fatalities, Environmental Cold Exposure



### H44 Leaving the Familiar: Suicidal Tourism in Cook County, Chicago, Illinois

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**Learning Overview:** After attending this presentation, attendees will have gained knowledge in suicidal tourism within Cook County, IL.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing data of a relatively rare phenomenon of suicidal tourism and the challenges this provides to death investigation, suicide prevention, and public health.

For many, suicide is a very personal and individualized act. Whether it is spontaneous or meticulously planned out, the action of taking one's life usually revolves around the familiar. It occurs at home, at work, or in a location the decedent is most comfortable with. In rare occasions, however, a person chooses to leave their immediate surroundings to commit suicide, in an act referred to as suicidal tourism.<sup>1</sup> Many who choose to travel head to larger cities and national landmarks. Places like the Golden Gate Bridge in San Francisco, CA, and the Grand Canyon National Park in Arizona are hotspots for those seeking to commit suicide away from home.<sup>2,3</sup> As a destination for suicidal tourism, Chicago and Cook County are different, as they lack well-known national landmarks, large national parks, or famous high bridges. What attracts people to Chicago to commit suicide is currently unknown. The goal of this investigation is to answer that question and look for epidemiologic and locational patterns to better support the public health mission of suicide prevention.

A retrospective search of the Cook County Medical Examiner's Office (CCMEO) electronic database, LABLynx ELab (LabLynx, Inc.), was undertaken. All cases where the manner was classified as suicide between 2014 and 2018 were collected and reviewed.

A total of 2,045 suicides were examined at the CCMEO over a five-year period (2014 through 2018). Of those, 4.6% (94/2,045) were committed by persons whose home address lay outside of Cook County. Persons with out-of-state addresses made up 1.9% (39/2,045) of total suicides. The leading five causes of death in descending order of frequency included jumping from height, self-inflicted gunshot wounds, hanging, train deaths, and drug overdose. Over half of the individuals travelled to Cook County from other parts of Illinois (59%; 55/94). Fewer travelled from out of state (41%; 39/94). No cases of international travelers were identified. Ages ranged between 17 and 75 years old, with an average age of 39. Most were identified as Caucasian (85%; 80/94), and the male-to-female ratio was 3.27. Common types of suicidal locations included hotel rooms, parking garages, train stations, and forest preserves.

For forensic pathologists, suicides present a unique challenge. Though the cause and manner of death may be relatively straightforward, the prevention of such deaths is not. The uniqueness of each suicide makes recommendations for prevention difficult to formulate. Studies such as the current one are crucial to provide data to help families, law enforcement, and medical professionals recognize individuals at risk of suicidal tourism and the common locations within Chicago that they frequent, in an attempt to prevent further loss of life.

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#### Suicidal Tourism, Suicide, Travel



### H45 Shallow-Water Blackout: A Rare Case of Death During Pool Free Diving

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**Learning Overview:** The goal of this presentation is to present a rare case of prolonged laryngospasm in a fit diver who was found submerged and lifeless on the bottom of a public swimming pool while he was training in free diving.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the usefulness of a multidisciplinary forensic approach by autopsy, histological/immunohistochemical, and toxicological investigation in cases of sudden and unexpected death in the water, particularly in all cases in which common signs of drowning are not found on the body.

The sudden and unexpected death of a young, fit diver in a public swimming pool represents a challenge to the forensic pathologist. The lack of internal and external drowning signs requires the consideration of a differential diagnosis that includes at least four syndromes (i.e., preexistent cardiac disease, abnormalities in electrical conduction, epilepsy, and hypoxic blackout). Hypoxic blackout can occur at any dive depth, including at constant depth, on ascent from depth, or at the surface following ascent from depth. “Shallow water blackout” is a term referring to the loss of consciousness that occurs when hypoxia is expedited by hypocapnia caused by voluntary hyperventilation before the dive, and where alternative causes of unconsciousness have been excluded.

During free diving, divers can rely only on the oxygen found in their lungs, blood, and tissues. Oxygen (O<sub>2</sub>) tends to decrease, while carbon dioxide (CO<sub>2</sub>) increases in the blood level, stimulating the brain centers associated with breathing. These impulses will induce an urgency to surface and to inspire, while powerful diaphragmatic contractions begin. This moment has been designated the “inspiration break point.” During voluntary diving, if a diver engages in prolonged presubmersion hyperventilation, the pre-dive CO<sub>2</sub> levels will fall and the subsequent CO<sub>2</sub> rise during submersion may not be sufficient to provoke the stimulus to surface before the O<sub>2</sub> blood levels fall and the individual loses consciousness. At this point, the inspiration break point occurs and the unconscious submerged victim is at high risk of drowning.

Presented here is a case report of a Caucasian, 32-year-old man, 171cm in length, found submerged and lifeless on the bottom of a swimming pool where he usually went to train. He had no family history of cardiovascular disease. He was retrieved after a few minutes, but cardiopulmonary resuscitation attempts were ineffective. A multidisciplinary forensic approach, including autopsy, histologic, and toxicologic investigation, was performed. The autopsy external examination showed no injuries. Internal examination, however, revealed subarachnoid hemorrhage and diffuse petechiae overlying the pleural surfaces of the congested and edematous lungs. Once sectioned and compressed, much serousanguinous fluid was extruded. The larger bronchial branches revealed foamy liquid contents. There were no gastric contents. Hematoxylin and Eosin (H&E) -stained histologic sections revealed polyvisceral stasis, thin cerebral hemorrhagic suffusion, and massive pulmonary edema. The toxicologic examination did not reveal drugs or alcohol in the serum. No diatoms were observed in either the water samples or the marrow.

In sum, these anatomic and pathologic findings would support a death attributable to a hypoxemic-hypercapnic condition induced by prolonged laryngospasm due to the apnea during immersion. The autopsy showed very little volume of liquid in the airways, but histologic evidence of acute pulmonary edema. The lack of ingested liquid would be compatible with a laryngeal spasm and cardiac arrest, the consequence of a voluntary respiratory arrest in prolonged apnea conditions.

#### Apnea, Sudden Death, Hypoxic Blackout



### H46 The Elphinstone Tragedy: Understanding the Chaos of a Human Stampede on the Staircase of a Railway Station Footbridge

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**Learning Overview:** After attending this presentation, attendees will understand the Elphinstone Tragedy, a human stampede and its classification, the clinical aspects of a stampede, the cause of death, and injury patterns in a stampede.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping to find the cause of death, understanding the mechanics of a stampede, and the reason behind it. This presentation will also be helpful to emergency medicine to clinically diagnose and treat survivors in a stampede. Additionally, this presentation will be impactful by analyzing the reasons behind such tragedy and the multidisciplinary approach to implement preventative measures.

Human stampedes are a major cause of mortality in mass gatherings, but they have received limited scientific attention. At approximately 10:30 a.m. local time on September 29, 2017, a stampede broke out at the Dussehra festival in Mumbai, India. The stampede arose on the staircase of a narrow footbridge of the Elphinstone railway station. Elphinstone railway station is located on the western railway lines and connects two of the major suburban lines in Mumbai. Ultimately, a total of 23 people were killed and 39 were injured. By approximately 11.30 a.m., 22 casualties had been transported to the King Edward Memorial (KEM) Hospital and 40 injured patients were admitted to the ward. Among those injured, one patient had sustained serious injuries and survived for 26 hours before expiring. The remaining victims sustained more minor injuries and were subsequently discharged. Of the total 23 casualties, 15 were men and 8 were women. On external examination, the most commonly injured body region was chest (19 cases), followed by lower limbs (15 cases), upper limbs (11 cases), head (8 cases), neck (3 cases), abdomen (2 cases), and spine (1 case). In 8 cases, there were associated rib fractures. The age group most commonly affected was 31–40 years old (7 cases), followed by 41–50 years old (5 cases), and 21–30 years old (4 cases.) In the Elphinstone Tragedy, mechanisms of death varied, including traumatic asphyxia (7 cases), crush injuries of the chest (7 cases), shock and hemorrhage due to vital organ injury (3 cases), head injury (3 cases), head injury associated with lung injury (1 case), blunt trauma of chest and abdomen (1 case), and positional asphyxia (1 case.)

Further investigation revealed that the incident occurred during the morning rush hour of the Dussehra festival at Elphinstone station when four trains arrived simultaneously at the station. It was raining at the time, and there were already numerous people crowded onto the staircase of a narrow bridge. It was postulated that someone may have slipped and fell when passengers resumed their travel after the rain, leading to the stampede; additionally, a rumor that the pedestrian bridge was collapsing may have caused the passengers to surge the staircase in order to flee.

A multidisciplinary approach to identifying crowded places and implementing appropriate preventive measures is needed. Prevention measures are related to planning for crowd management and venue design, with review of their effectiveness. Drills are recommended in the preparedness phase to improve coordination and communication. Delays in decisions, poor triage, and/or loss of medical records are common problems in the response phase and may lead to worsened outcome. Broad footbridges and deployment of security guards to the platforms of crowded places are a few recommended measures to prevent this type of tragedy from occurring in other developing countries in the future.

#### **Elphinstone, Stampede, Railway Station**

## H47 A Unique Pattern of Tusk Injuries by Wild Boar: A Ten-Year Autopsy Analysis

Nilesh Keshav Tumram, MD\*, Department of Forensic Medicine and Toxicology, Nagpur, Maharashtra 440013, INDIA

**Learning Overview:** After attending this presentation, attendees will be able to identify the uniqueness of injuries caused by the tusk of wild boar, which may be of a distinct type.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by creating awareness of another unique form of injury that may be of medicolegal significance if such a case is found in deserted places with no witness to the incident. This presentation highlights these type of injuries, which are rarely mentioned in the available literature.

Wild boar is a common name for various species of the pig of the genus *Sus*, part of the biological family *Suidae*. It is one of the most common and widespread large mammals in the world.<sup>1</sup> The body of the wild boar is compact, with a large head and relatively short legs. The fur consists of stiff bristles and usually additional finer fur.<sup>2</sup> The average weight and length of a wild boar are 50 to 90kg and 120 to 180cm, respectively. After two years of age, male wild boars grow tusks from both the upper and lower canines that curve upward. The lower tusks are extremely sharp and usually measure 6cm in length. These tusks serve as weapons during fighting. Wild boars are primarily nocturnal animals and are naturally timid; therefore, confrontations between wild boars and humans are typically very rare.<sup>3</sup>

Recently, however, extinction of natural wild boar enemies (e.g., tigers, wolves, bears, louboutins, leopards, and lynxes) coupled with their high reproduction rates and their ability to adapt to a variety of environments have led to an increase in the wild boar population.<sup>4</sup> They are potentially dangerous animals due to their razor-sharp tusks and may cause serious injuries if confrontations do occur. Wild boars are known to be more aggressive during the mating season and when cornered.<sup>5</sup> It is suspected that wild boar attacks are underreported, and forensic pathologists may be unfamiliar with the pattern of the wild boar tusk injuries as the features are rarely described in the literature.<sup>5-8</sup>

The present study characterizes the mechanism of injuries caused by wild boar attacks. This presentation also describes the specific pattern of injuries over the body which seem to be unique to wild boar attacks. The size and shape of injuries caused by wild boar are very specific and identifiable, even in the absence of a witness, making it important in medicolegal autopsy cases with limited or inadequate history. This presentation will also review the importance of having such knowledge in order to accurately certify such deaths in medicolegal cases.

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### Wild Boar, Tusk Injuries, Injury Pattern



### H48 Hypothermia Deaths and Altered Mental Status

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**Learning Overview:** After attending this presentation, attendees will understand classic patterns that occur among hypothermia deaths related to altered mental status, as well as be able to consider future methods to reduce mortality from hypothermia in these populations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting some of the inherent risks of hypothermia deaths related to altered mental status. Understanding these risks will set the stage so that preventive strategies may be further developed and implemented with the goal of reducing mortality within specific vulnerable populations.

Much of the United States experiences sub-freezing winters. While most people who have access to shelter would find themselves apt to seek shelter and safety indoors, those who are cognitively impaired have difficulty eliciting or acting on this reflexive behavior.<sup>1</sup>

Hypothermia is a state in which core body temperature is approximately 33°C or below. Hypothermia from environmental exposure to the cold is termed “primary hypothermia,” and it can result in either focal or systemic injury. The risk of death from hypothermia is noteworthy and particularly high during prolonged exposures to sub-freezing temperatures, though it may also occur in higher temperatures or even indoors.<sup>2</sup>

This presentation provides a review of multiple cases of lethal hypothermia where the deaths were related to some form of altered mental status. Various causes of impaired judgment found include, but are not limited to, dementia, physiologic hormone or electrolyte imbalance, and drug use or abuse.<sup>1</sup> The context of each case differed from the others, but several classic morphologic stigmata of hypothermia were commonly seen, including gastric Wischnewski spots and “frost erythema.” Paradoxical undressing was another relatively common finding.

Among others, altered mental status is a well-known risk factor for hypothermia. Common causes of altered mental status include substance use as well as degenerative neurologic disorders, including various forms of dementia.<sup>1</sup> Though each examined death in this study was related to hypothermia, many of the decedents’ comorbidities often had coexisting physical manifestations; these may have played a contributing role in these deaths.

Hypothermia appears to be responsible for the deaths of seemingly healthy people who are unable to obtain help after being accidentally exposed to cold temperatures, especially in the winter in the Midwestern United States. While acute circumstances of altered mental status may appear to be the triggering event for the examined cases, it is critical to understand the decedents’ preexisting diseases. Considering comorbidities, such as atherosclerosis, respiratory disease, alcohol misuse, or metabolic disturbances, can provide insight to the inappropriate thought process and inability to seek shelter in cold conditions, which ultimately can lead to loss of consciousness and death.<sup>2</sup>

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**Hypothermia, Freezing, Impaired**



### H49 An Evaluation of Sudden Deaths Due to Myocarditis: A Study of Autopsy Cases

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**Learning Overview:** After attending this presentation, attendees will better understand the characteristic features of acute myocarditis as a cause of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance of forensic autopsy examination with thorough histological study on all sudden unexpected deaths and by understanding the challenges to forensic pathologists when diagnosing acute myocarditis.

Causes of sudden cardiac deaths have been widely reported with limited data focused specifically on myocarditis. Myocarditis is an inflammatory disease of the heart that can cause sudden and unexpected deaths in otherwise healthy individuals. Cases were selected for this study with the following criteria: (1) sudden and unexpected deaths; (2) autopsied cases with complete records, including death scene investigation report and available medical records, autopsy report, toxicology report, and (3) myocarditis was the primary cause of death. The following information was extracted for each case: (1) demographic data, including age, race, and gender; (2) investigation report, including the circumstances of death and known medical history prior to death; (3) autopsy findings; and (4) toxicology report.

This retrospective review of cases from a statewide Office of the Chief Medical Examiner (OCME), MD, yielded a total of 103 sudden unexpected deaths due to myocarditis (1.7% of all natural deaths) in the past ten years. Of the 103 cases, 58 were male and 45 were female (male:female=1.3:1); 37 were African American and 51 were Caucasian. The mean age at death was 31±17 years, with a median age of 30 years. Of the 103 cases, 45 (43.7%) patients were witnessed collapsed. Conditions surrounding death were recorded in 66 cases, with 34 deaths occurring during sleeping or resting. Twenty-six deaths occurred during normal daily activity, such as walking, working, or watching television. Four deaths occurred during exertion, such as exercising at the gym or heavy physical work, and 2 deaths were associated with emotional stress. Eight-six (83.5%) individuals reported chest pain, nausea, or palpitations during a time period less than one day to more than two weeks prior to their deaths. None of the patients sought medical attention. Common cardiac macroscopic findings included ventricular dilatation (39.8%), mild coronary stenosis (17.5%), mottled myocardial appearance (15.5%), and myocardial fibrosis (10.7%). The histological classification of myocarditis was based on the predominant type of inflammatory cell infiltration. In this study group, lymphocytic myocarditis was most common, accounting for 56 cases (54.4%), followed by neutrophilic (32 cases, 31.7%), eosinophilic (13 cases, 12.6%), and giant cell type (2 cases, 1.9%). Microscopic examination revealed myocyte necrosis in 69 cases (67.0%) and interstitial or perivascular fibrosis in 48 cases (46.6%). The percentage of myocyte necrosis was 75.0% (42/58 cases) in lymphocytic, 65.6% (21/31 cases) in neutrophilic, 30.8% (4/13 cases) in eosinophilic, and 100% (2/2 cases) in giant cell myocarditis.

Determination of myocarditis as the cause of death continues to present a major challenge to forensic pathologists because histopathologic findings can be subtle and the diagnosis of myocarditis remains difficult. In cases of sudden unexpected death, especially sudden death in young people, forensic pathologists should consider myocarditis as possible cause. Representative sections from the anterior, lateral, and posterior wall of the atria and ventricles and sections from the ventricular septum should be submitted for microscopic examination.

#### Acute Myocarditis, Sudden Death, Forensic Autopsy





### H50 A Case of Pulmonary Artery Dissection in a Woman With Chronic Pulmonary Hypertension

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**Learning Overview:** After attending this presentation, attendees will better understand pulmonary artery dissection as a fatal complication of chronic pulmonary hypertension and its autopsy findings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of sudden death in adults with long-standing pulmonary hypertension.

A 57-year-old woman with a history of pulmonary hypertension and a remote history of polysubstance abuse (1998) was found unresponsive in her locked residence by a neighbor after reportedly feeling unwell for a couple days.

At autopsy, external examination noted digital clubbing. Opening the chest cavity revealed a tense, discolored pericardial sac containing 650mL of liquid and clotted blood. Hemorrhage was identified at the root of the pulmonary artery, and careful examination revealed a 2mm full thickness tear, located 5mm distal to the pulmonic valve. The dissection extended 6.0cm distally to the left main pulmonary artery, terminating 5mm proximal to the take-off of the apicoposterior and anterior segment branches. The pulmonary trunk was dilated, measuring 3.8cm in diameter at the pulmonary root, 3.8cm at the left main pulmonary artery, and 3.2cm at the right main pulmonary artery. There was diffuse atherosclerosis extending into the distal branches of the pulmonary artery. The heart weighed 530 grams and displayed right ventricular hypertrophy with a wall thickness of 8mm, as well as dilated tricuspid, pulmonic, and mitral valves (12.5cm, 9.3cm, and 11.0 cm, respectively).<sup>1</sup> Multiple cavernous hemangiomas of the liver ranging in size from 5mm to 35mm were incidentally identified.

Histologically, the pulmonary artery showed extensive medial degeneration with a large defect extending from the tunica media into the adventitia with associated hemorrhage. The small and large pulmonary vessels showed medial hypertrophy, highlighted with elastin stain. Scattered plexiform lesions were identified. Elastin stain further highlighted fragmentation and loss of elastic fibers with increased intralamellar and translamellar extracellular mucoid material noted on Alcian blue stain. The aorta showed intact elastic fibers with no increase in extracellular mucoid material.

While right ventricular failure is the main cause of death in patients with pulmonary hypertension, autopsy is rarely performed in these cases of sudden death in otherwise stable patients. It has been reported that 28% of pulmonary artery hypertension patients die unexpectedly within three years of diagnosis. Dilatation of the pulmonary artery has been independently associated with unexpected death in these patients with two possible mechanisms: pulmonary artery rupture/dissection and potentially arrhythmogenic pulmonary artery compression of the left main coronary artery. In one study, 2 of 26 unexpectedly deceased pulmonary hypertension patients underwent autopsies, and both showed cardiac tamponade due to pulmonary artery dilatation and dissection.<sup>2</sup>

Pulmonary artery dissection is a rare, but fatal, complication of chronic pulmonary hypertension. Some of the underlying etiologies include: congenital cardiac diseases, pulmonary artery obstruction due to emboli (thrombotic, tumor or foreign material), fibrosing pulmonary diseases, emphysema and left-sided cardiac conditions. If there is no underlying etiology discovered, a diagnosis of primary or idiopathic pulmonary hypertension is made. Histologic findings are often most pronounced in small pulmonary arteries and include medial thickening due to smooth muscle hyperplasia and hypertrophy, intimal thickening due to intimal smooth muscle cells or fibrosis, plexiform lesions, and dilation lesions.<sup>1</sup>

Overall, pulmonary artery dissection as a result of chronic pulmonary hypertension is a rare but fatal entity that may be seen in the forensic pathology community.

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#### Pulmonary Hypertension, Pulmonary Artery Dissection, Hemopericardium



### H51 Mal D’Afrique: The Mysteries of Endomyocardial Fibrosis in Western Countries

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**Learning Overview:** After attending this presentation, attendees will be aware of a very uncommon cause of death in Western countries: endomyocardial fibrosis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the features of an uncommon cardiac disease that is rather endemic in African men under the age of 30 years old.

Endomyocardial fibrosis is caused by fibrous thickening of the endocardium, involving one or both ventricles and the atrioventricular valves. Its first description dates back to 1948 in Uganda. Epidemiologic studies report endemic prevalence in Sub-Saharan Africa and other tropical areas. For Western countries, such as Italy, however, endomyocardial fibrosis is rare and somewhat mysterious in terms of clinical manifestations, etiology/pathophysiology, diagnosis, and therapeutic management.

This report presents the case of a 21-year-old man (height 166cm, weight 50kg) from the Republic of Gambia in West Africa who suddenly collapsed at a center for asylum seekers located in Apulia, in Southeastern Italy. One week earlier, he had presented to a local physician with high fever, chest pain, and asthenia; he was prescribed antibiotic therapy for a presumed influenza infection. The man was unable to be resuscitated and was pronounced dead. A judicial autopsy was requested. External examination was notable for Janeway spots, cachexia, and the absence of traumatic injuries. The heart weighed 350g and showed a slightly increased shape and volume. Coronary arterial sectioning revealed subcritical stenosis of the left coronary artery that was complicated by a small thrombus. The right coronary artery was patent. The right ventricular wall was partially substituted by fibrosis; the left ventricle likewise revealed fibrotic remodeling. Additionally, the left ventricular apex was completely occluded by endoluminal vegetation. Histologic examination confirmed that the left cardiac ventricular wall was replaced by diffuse endomyocardial fibrosis associated with an inflammatory infiltrate consisting of lymphocytes, plasma cells, and a rich eosinophilic component that was surmounted by a large vegetative fibrinous thrombus in the initial phases of organization. No histologic evidence of acute ischemia was detected. In accordance with these medicolegal investigative results, the cause of death was attributed to endomyocardial fibrosis leading to sudden cardiac death.

In Western countries, endomyocardial fibrosis was previously considered an “obscure” or “obsolete” pathology, scarcely investigated and without specific therapies. The clinical signs are not easy to evaluate, and this makes it difficult to determine the correct diagnosis clinically and, consequently, to administer appropriate therapy. The recent increase of this disease in Western countries is closely linked to migratory flows. According to the United Nations High Commissioner for Refugees (UNHCR) data, 119,247 refugees arrived in Italy between January 1–December 31 in 2017. For this reason, in industrialized countries like Italy, endomyocardial fibrosis—along with other previously obscure cardiovascular diseases such as Chagas disease—now represent novel and emerging diseases.

Currently, there is an epidemiologic transition occurring in which cardiac diseases previously deemed obscure at certain latitudes are now on the rise due to increasing numbers of immigrant citizens. Health care professionals—including forensic pathologists—are thus advised to develop familiarity with the features of these emerging cardiomyopathies. Creating a registry of rarer cardiac entities aimed at increasing knowledge of the diseases as well as diagnostic and therapeutic strategies is suggested.

#### **Endomyocardial Fibrosis, Cardiovascular Diseases, Restrictive Cardiomyopathy**



## H52 Emphysematous Gastritis: A Rare Disease With a Fulminant Course

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**Learning Overview:** After attending this presentation, attendees will understand features and clinical history distinguishing emphysematous gastritis from common mimics at autopsy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the risk factors, casual organisms, and mortality associated with emphysematous gastritis. This presentation will assist the community overall by increasing awareness of and aiding in the diagnosis of this infectious condition at autopsy.

**Background:** Emphysematous gastritis is a gastric wall infection with intramural pneumatosis suspected to arise due to microbial gas production. It has a fulminant course and significant mortality. Due to the relative rarity, limited published literature exists. Additionally, the disease may be underreported as it can be mistaken for other entities, such as gastric emphysema (i.e., gastric pneumatosis without underlying infection) or postmortem decompositional change.

**Methods:** Autopsy reports from the University of Wisconsin Madison School of Medicine's electronic database between 2003 and 2018 were searched utilizing the term "emphysematous gastritis." All retrieved cases were reviewed. A systematic PubMed® literature search utilizing the same search terms with limits "English language" and "human subjects" between 2000 and 2018 was also conducted. Data on patient demographics, causal pathogens, and outcomes were abstracted.

**Results:** A single case of emphysematous gastritis was diagnosed via postmortem examination at the University of Wisconsin Madison School of Medicine over the preceding 15 years (i.e., a 60-year-old woman undergoing chemotherapy for metastatic mucinous adenocarcinoma suspected to be of gastrointestinal origin). Three days prior to death, she presented with intractable nausea and vomiting that quickly progressed to diffuse abdominal pain. X-ray revealed marked gastric distension with probable gastric pneumatosis but no definitive pneumoperitoneum. She became hemodynamically unstable and expired. Autopsy was performed the day after death and revealed a primary gallbladder carcinoma. The gastric mucosa appeared markedly dusky and hemorrhagic with a palpable crepitation of the wall. No discrete gastrointestinal tear or perforation was evident. Microscopic sections revealed vacuoles consistent with gas bubbles extensively involving the gastric wall as well as admixed bacterial organisms that were predominantly gram-negative rods by gram staining.

Fifty-nine additional cases of emphysematous gastritis were identified in the literature via PubMed® search. Analysis showed a mean age at diagnosis of 50.5 years (range: 2–94 years). There was a slight male predominance (1.3:1). Greater than half of patients were immunocompromised (34/60; 56.7%). Other risk factors included peptic ulcer disease (11/60; 18.3%), history of abdominal surgery (11/60; 18.3%), caustic ingestion (9/60; 15%), and malignancy (7/60; 11.7%). Multiple risk factors were present in nearly half of the cases (28/60; 46.7%), with lesser numbers having a single risk factor (24/60; 40%) or no known risk factors (8/60; 13.3%). Causal pathogens were identified in 45% of cases. Most commonly, these were bacterial only (14/60; 23.3%) or polymicrobial (9/60; 15%); however, a few were fungal only (4/60; 6.7%). In more than half of the cases, infectious disease testing was either not reported (23/60; 38.3%) or else was reportedly negative (10/60; 16.7%). The overall mortality of emphysematous gastritis was 33.3% (20/60). Malignancy ( $n=7$ ) portended a worse prognosis, with a mortality of 42.9%. No cases of emphysematous gastritis associated with gallbladder carcinoma were reported.

**Conclusions:** This presentation reports the first case of emphysematous gastritis arising in association with gallbladder adenocarcinoma. As in this case, the clinical presentation with emphysematous gastritis is typically fulminant with symptoms such as nausea/vomiting, abdominal pain, and hematemesis. Gastric emphysema, conversely, is typically asymptomatic, arises due to disruption in gastric mucosal integrity leading to air entry, and resolves spontaneously. Given its high mortality, pathologists should become familiar with the epidemiologic and histopathologic features of emphysematous gastritis, as well as how to distinguish it from common mimics. Autopsy should be encouraged in all suspected cases to improve understanding of the disease pathogenesis.

### Forensic Autopsy, Emphysematous, Gastritis



## H53 Cardiac Amyloidosis—Two Cases

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**Learning Overview:** After attending this presentation, attendees will better understand the genetic and pathologic differences between various forms of cardiac amyloidosis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting two cases of cardiac amyloidosis that were diagnosed at autopsy.

Reported here are two cases of death caused by amyloidosis with cardiac involvement. One case involved a 71-year-old Vietnamese woman. Autopsy revealed severe cardiac interstitial fibrosis/amyloidosis, with associated cardiomegaly (470 grams), as well as focal, microscopic amyloidosis within the stomach, liver, and kidney. Mass spectrometry of cardiac tissue was consistent with transthyretin-type amyloid deposition. Transthyretin sequencing expressed a mutation (Ala97Ser) indicative of hereditary transthyretin amyloidosis.

The second case involved a 77-year-old male with a past medical history most significant for congestive heart failure and clinical work-up concerning amyloidosis. Autopsy revealed diffuse cardiac amyloidosis, with associated cardiomegaly (830 grams), dilated heart, and biventricular hypertrophy, as well as microscopic amyloidosis within the liver and kidneys.

Heart disease is the most common cause of death in the United States, with the majority of cases attributable to ischemic and hypertensive heart disease. More rare are the infiltrative cardiomyopathies, which includes amyloidosis.<sup>1</sup> Cardiac amyloidosis may be cardiac-limited or have associated extracardiac involvement, and is nearly always caused by one of two proteins: immunoglobulin light-chain or transthyretin.<sup>2</sup>

Amyloid Light-chain (AL) amyloidosis is the most common type of pathologic cardiac amyloidosis and usually occurs in the setting of plasma cell dyscrasias.<sup>3</sup> Amyloid Transthyretin (ATTR) amyloidosis can be due to wild-type or mutated (hereditary) transthyretin. Wild-type ATTR (previously senile) amyloidosis is the second most common cause of cardiac amyloidosis and occurs mostly in elderly men. Hereditary ATTR amyloidosis is less common, presents at a younger age, and has various geographic distributions.<sup>4</sup> Mortality from cardiac amyloidosis is often due to heart failure or fatal arrhythmias.<sup>5</sup>

The cases serve to highlight cardiac amyloidosis in the forensic autopsy setting. Forensic pathologists need to be aware of the various forms of cardiac amyloidosis and the types of postmortem testing available to better characterize the diagnosis. Depending on the type of amyloidosis, notification of surviving family members may be appropriate, as some cases have a familial tendency.

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### Cardiac Amyloidosis, Infiltrative Cardiomyopathy, Transthyretin



### H54 An Anomalous Origin of Right Coronary Artery as a Possible Cause of Sudden Cardiac Death in an Athlete: A Case Report

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**Learning Overview:** After attending this presentation, attendees will better understand an anomalous origin of right coronary artery, a rare condition that can be asymptomatic, but can also lead to sudden cardiac death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a cause of cardiac sudden death in athletes and the potential legal repercussions for the physicians that were responsible for the medical follow-up.

Sudden cardiac death of athletes is a source of confusion for the relatives as well as the general public, not only because it affects young individuals, but also because practicing sports is generally considered to be associated with an improved quality of life.

**Case Report:** An 18-year-old male was found dead in his bed by his roommates. He was a young professional soccer player and had been an intern in a soccer training center for two years. He had no significant medical history. At external examination, no signs of trauma were found. The body weighted 70kg with a length of 181cm. At autopsy, the organs were congested. The heart weighted 383g and showed an anomalous origin of the right coronary artery. The right coronary artery arose 1.5cm above the right coronary sinus. There was a small bridge of tissue that recovered the origin of the artery. The right coronary then made an angulation before assuming the expected path in the coronary sulcus. The rest of the autopsy was unremarkable. Histology confirmed the right coronary arterial anomaly. As a result of this anomaly, it was suspected that the death could have been due to a temporary interruption of blood flow leading to myocardial ischemia. Toxicologic analysis was negative. After the autopsy, the medical charts of the deceased were analyzed. An electrocardiogram and echocardiography had been performed upon the arrival of the male at the training center, as well as a cardiac stress test. He had another complete examination one year later. These repeated evaluations met the criteria that are required by French law for professional athletes and were strictly normal.

**Discussion:** The prevalence of anomalous origin of the right coronary artery is estimated at 0.25% in the literature. It is an infrequent congenital anomaly. The right coronary artery originates either from the left coronary sinus or from the aortic wall above the coronary sinus, as in this case. The patient may be asymptomatic, but this condition may also lead to myocardial infarction, angina pectoris, or sudden death. In this case, it was important to establish that this type of anomaly cannot be detected by standard echocardiography, so that the medical center that evaluated the health of the athletes was not legally responsible.

**Forensic Science, Sudden Cardiac Death, Anomalous Origin of Coronary Artery**

## H55 A Tell-Tale Heart: A Case of Takotsubo Cardiomyopathy at Autopsy

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**Learning Overview:** After attending this presentation, attendees will recognize the clinical history of Takotsubo Cardiomyopathy (TCM) and be familiar with its pathological findings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing attention to a rare cardiomyopathy that, while pertinent to a medicolegal investigation, may go unrecognized outside of a hospital setting.

**Background:** TCM, also known as stress cardiomyopathy, is an acute and transient left ventricular wall-motion abnormality involving the apex and mid portions of the ventricle while sparing the base, which can simulate acute coronary syndrome in the absence of significant coronary artery stenosis.<sup>1,2</sup> It is often preceded by emotional or physical stress.<sup>1,3</sup> Although the precise mechanism behind this contractile dysfunction remains elusive, it is thought to be due to a catecholamine excess resulting in microcirculatory dysfunction and direct myocardial toxicity.<sup>1,4</sup>

**Case Report:** A 51-year-old female with Type 2 diabetes mellitus, end-stage renal disease on hemodialysis, and severe peripheral arterial disease was admitted with a gangrenous wound of the hand and concern for osteomyelitis, as well as altered mental status and septic shock. Despite amputation of the hand and broad-spectrum antimicrobial treatment, she became increasingly hypotensive, with rising lactate. High-sensitivity Troponin T was 173ng/L on hospital day three, eventually peaking at 328ng/L on hospital day four before trending downward over the course of hospital day five (reference range: <14ng/L). Serial electrocardiograms demonstrated ST abnormality and inverted T waves, concerning for ischemia. An echocardiogram on hospital day four demonstrated a significant large apical wall motion abnormality of the left ventricle, extending to mid segments, with hyperkinesis of the basal segments. These findings differed dramatically from the previous day's echocardiogram, and were suspicious for TCM. The patient's condition worsened, and she developed unstable ventricular arrhythmias, which progressed into Pulseless Electrical Activity (PEA) arrest. Per her code status, no aggressive resuscitation was initiated, and she expired on hospital day five.

**Pathological Findings:** At autopsy, the heart weighed 269g, with a moderate amount of epicardial fat. The coronary arteries demonstrated minimal to mild atherosclerosis, with up to 40% stenosis in the left anterior descending artery. There was no hemorrhage, fibrosis, or other evidence of infarct. The mitral valve was modestly dilated at 11.5cm (average: 8–10.5cm). Histologically, the myocardium demonstrated striking myocyte hypertrophy with "boxcar nuclei," present throughout the bilateral atrial and ventricular free walls and interventricular septum. Interstitial fibrosis was also present, with scattered mononuclear cell infiltrates and contraction bands. Immunohistochemical staining demonstrated these mononuclear cells to be largely CD68 positive macrophages, with scattered single CD3 or CD20 positive lymphocytes. Staining with C4d demonstrated scattered foci of myocyte necrosis. The left ventricular free wall demonstrated fibrinous pericarditis with a CD3 positive lymphocytic infiltrate. Geographically, the left ventricle was the most severely affected, with the base more involved than the apex. The right atrium was more involved than the left atrium.

**Discussion:** In this case, TCM was preceded by significant physiologic stress (infection, septic shock, amputation). The patient's Electrocardiogram (ECG) findings were non-specific, as is usual in 85% of TCM cases.<sup>2</sup> Troponin T was markedly elevated, which, while not typical for TCM, has been reported.<sup>3-5</sup> The clinical and radiological features of TCM are well-documented, but the pathological findings are less so, as TCM is largely a clinical diagnosis.<sup>1,2</sup> Described microscopic findings include myocyte hypertrophy, C4d positive myocyte necrosis, interstitial fibrosis, mononuclear cell infiltrate, and contraction bands with or without necrosis, the likes of which are present in the current case.<sup>1,2,4,5</sup> Despite the generally good prognosis, complications of TCM include heart failure, thromboembolism, ventricular arrhythmia, and ventricular free wall rupture.<sup>2</sup>

**Conclusion:** TCM is a left ventricular wall-motion abnormality often precipitated by emotional or physical stress, and thought to be due to an excess of catecholamines resulting in myocyte toxicity and microcirculatory dysfunction. In spite of an overall good prognosis, TCM is not a harmless entity. Recognition of the clinical presentation, complications, and pathologic findings is important for death investigation.

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### Takotsubo, Cardiomyopathy, Autopsy



### H56 Autopsy Findings of Individuals Displaying Symptoms of Pica

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**Learning Overview:** After attending this presentation, attendees will be able to visually understand the physical complications of an individual who suffers from a mental disorder known as pica. Pica is an eating disorder that involves the individual craving and eating items that are not generally thought of as food and contain no nutritional value. Some of these items may include carpet fibers, hair, dirt, and other common household items. Pica is considered a dangerous and often deadly illness if not monitored due to the likelihood of intestinal blockages or perforations from the toxicity of the items that are sometimes consumed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by displaying three cases of patients that displayed pica symptoms at the Mississippi State Medical Examiner's Office for autopsy and by exhibiting the pathological findings of each case.

**Case 1:** A 2-year-old, White male was found unresponsive in his playpen. His mother stated that he had been pulling at his ears and throat for some time before he was found. He had no known medical history and, other than not feeling well the past few days, was considered a healthy child. However, the victim's mother also stated that she had recently reached out for medical attention regarding her son missing milestones, such as walking and talking. At autopsy, the child's abdomen was severely distended, and he showed multiple lacerations to his face and mouth. Internal examination of the stomach revealed that the child had developed a bezoar due to his ingestion of foreign materials. These materials seemed to primarily include items such as carpet fibers, jean fibers, and hair.

**Case 2:** A 31-year-old, White male was transported to the hospital from prison after complaining of abdominal pain. The patient had no known significant medical history but had been anecdotally reported to eat the toothpaste tubes from the prisoner hygiene kits. At autopsy, examination of the gastric contents revealed multiple non-edible items including paper towels, toothpaste tubes, and apple juice cartons. The victim's ultimate cause of death was peritonitis from a bowel obstruction.

**Case 3:** A 19-year-old, African-American male was a patient at the state mental hospital and had previously been diagnosed with pica. During a transportation to facilities, the victim vomited what was thought to be fecal matter along with a latex glove. It was believed that the glove had become lodged in his airway for too long, and he later died from the incident. Upon internal examination at autopsy, it was found the victim's gastrointestinal tract contained items such as pens, plastic bags, latex gloves, and coins.

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#### Pica, Mental Disability, Foreign Material



### H57 Unexpected and Sudden Cardiac Death Due to Eosinophilic Myocarditis (EM): Still a Difficult Task for Forensic Pathologists

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**Learning Overview:** The goal of this presentation is to highlight that that antemortem diagnosis of EM is difficult due to ambiguous and non-specific symptoms. Although predisposing factors exist, EM may occur in healthy persons with no history of hypersensitivity. Death due to EM could be avoided through a prompt diagnosis and the administration of appropriate therapy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the importance of exhaustive and detailed cardio-pathological analysis in cases of sudden and unexpected death. The anamnestic analysis (when possible) is also fundamental to track down any prodrome or predisposing factors. Although infrequent, EM must always be considered in cases of sudden death, even in young subjects and in the absence of specific symptoms.

In this presentation, a rare case of unexpected death caused by an undiagnosed and undetected EM is described. This topic is still controversial, even though myocarditis represents one of the main causes of sudden cardiac death. The criteria for the diagnosis of EM are debated in the forensic science community, and no standard protocol has been agreed upon.

**Case Report:** A 52-year-old man, in apparent good health, suddenly died while he was working as an accountant. According to the medical history, he suffered a mild temperature and diarrhea about ten days before death; however, the symptoms had disappeared after two to three days. He had no previous history of chronic or autoimmune pathologies, allergies, drug abuse, parasitic infections, or recent vaccinations.

**Autopsy Findings:** The external examination was unremarkable. The macroscopic heart examination showed no significant signs of hypertrophy. The coronary arteries were patent and free of moderate/severe atherosclerosis. The myocardium appeared flaccid, dilated, and pale. The lungs were edematous and congested. There were no other macroscopic pathological findings.

**Microscopic Examination:** Hematoxylin and eosin-stained histologic sections showed inflammatory myocardial damage of the subepicardial and mid-mural region of the right ventricle and the anterior and posterior wall of the left ventricle. The inflammatory myocardial infiltrate consisted of many eosinophils (partially degranulated), macrophages, and a few neutrophils and T lymphocytes. Immunohistochemical analysis showed largely CD15+ and CD68+ cells. Toxicologic investigations did not reveal the presence of drugs or substances of abuse.

**Discussion and Conclusion:** In this case, a complete forensic approach led to the conclusion that death was caused by a fatal arrhythmia related to the extensive myocardial inflammatory damage (EM). The World Health Organization (WHO) defines myocarditis as “an inflammatory disease of the heart muscle, diagnosed by established histological, immunological and immunohistochemical criteria.” EM is an uncommon but potentially lethal type of myocarditis. The clinical as well as postmortem diagnosis of EM is difficult and often underrated. A comprehensive cardio-pathologic investigation is mandatory for a postmortem diagnosis of EM. Extensive myocardial sampling circumferentially, including the left and right ventricles in transverse section (as advised by the Association for European Cardiovascular Pathology guidelines), is always required.<sup>1</sup>

The histology of EM changes significantly depending on the extent and composition of inflammation, as well as associated myocardial damage. Necrosis of myocardial cells can be absent, focal, multifocal, or rarely extensive. Eosinophils can be preponderant or be a portion of mixed inflammatory cells with macrophages, plasma cells, lymphocytes, and even neutrophils. The role of the forensic pathologist is particularly important in correctly identifying EM at autopsy, particularly in cases that have remained undiagnosed antemortem. Because symptoms are non-specific and underestimated, the diagnosis is often not made until the time of autopsy. On completing this presentation, attendees will have gained knowledge of EM, including how a complete forensic investigation is essential to ascertain the cause of death.

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#### Eosinophilic Myocarditis, Sudden Cardiac Death, Autopsy





## H58 An Unusual In-Custody Death

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**Learning Overview:** After attending this presentation, attendees will have learned of an unusual death in jail and more considerations for the investigation of the “in-custody” death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a complicated case with the decedent’s psychological behaviors and secondary adrenal insufficiency that could have been the cause or result of one another.

In-custody deaths are invariably complex and high-profile cases. They require thorough investigation of the scene, including acquisition of any videos of the scene, the decedents’ behaviors prior to the death, eyewitness’ statements, and the circumstances surrounding the event. It is also important to get the decedents’ medical histories and perform complete autopsies with toxicologic studies.

The case of a 27-year-old White male found unresponsive in his jail cell is presented. His medical history was significant for bipolar disorder and borderline personality disorder. He also suffered from panhypopituitarism due to resection of his pituitary for a craniopharyngioma while still a child. He was being treated with desmopressin but had recently indicated that he was on a hunger strike, which included refusing water, food, and medications. Following transport to the hospital he was diagnosed with hypoglycemia, electrolyte insufficiencies, dehydration, and shock. He ultimately expired and an autopsy was requested. Autopsy findings included replacement of the pituitary gland by a 1.5cm greenish fluid-filled cyst. However, residual pituitary tissue was identified microscopically. The right adrenal gland was not identified, and the left adrenal gland was markedly atrophic (4.4 grams), as was the thyroid gland (10 grams). Other significant findings included bilateral pleural effusions (500ml on each side) and ascites (500ml).

Secondary adrenal insufficiency occurs when the pituitary gland fails to produce enough Adrenocorticotropic (ACTH), a hormone that stimulates the adrenal glands to produce the hormone cortisol.<sup>1</sup> If ACTH output is too low, cortisol production drops. Eventually, the adrenal glands can shrink due to a lack of ACTH stimulation. The etiology of secondary adrenal insufficiency may include stoppage of corticosteroid medication and surgical removal of pituitary tumors; other less common causes include tumors or infections of the pituitary gland, loss of blood flow to the pituitary gland, exposure of the pituitary gland to radiation, or the surgical removal of parts of the hypothalamus.<sup>2</sup> The slowly progressing symptoms of adrenal insufficiency can rapidly evolve into an adrenal crisis with any additional stresses.<sup>3</sup> The symptoms of adrenal crisis include dehydration, low blood pressure, loss of consciousness, and death. In the current presented case, it is suspected that adrenal crisis likely contributed to and/or was exacerbated by the grave consequences of the decedent’s hunger strike with resultant hypoglycemia, electrolyte insufficiencies, and dehydration.

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### Panhypopituitarism, Adrenal Crisis, In-Custody Death

### H59 A Case of Fulminant Spontaneous Necrotizing Soft Tissues Infections (NSTI) of the Chest Wall in a Man With No Risk Factors

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**Learning Overview:** After attending this presentation, attendees will be aware of the case of a patient with no risk factors who developed a mono-microbial spontaneous NSTI of the chest wall that was rapidly lethal.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting that NSTI of the chest wall, usually described in patients with pleural empyema or with a chest tube, can also occur spontaneously, in the absence of risk factors. Moreover, this presentation will impact the forensic science community by highlighting that a fulminant mono-microbial NSTI can occur in an immunocompetent patient.

NSTI refers to infection of any layer within the soft tissue compartment with associated necrotic changes.<sup>1,2</sup> It can be caused by different types of bacteria, including *Bacteroides*, *Streptococcus*, *E. coli*, and *Clostridium*—among many others.<sup>3</sup> The infections are usually polymicrobial, but may be monomicrobial in immunosuppressed patients. NSTI are most commonly described as occurring in the extremities, perineum (i.e., Fournier gangrene), abdomen, and head/neck.<sup>4-6</sup> Thoracic NSTI are extremely rare; the few cases reported in the international literature describe patients who have undergone thoracic surgery or had pleural empyemas.<sup>7</sup>

The case of a 67-year-old man who developed spontaneous NSTI of the chest wall following minimal muscle strain and in the absence of other risk factors is reported. He was admitted to the Emergency Room (ER) for moderate back pain radiating from the 3<sup>rd</sup>-4<sup>th</sup> right ribs. Clinical examination described a hematoma. He was discharged with prescribed painkillers but returned a few hours later for therapy-resistant pain. Clinical examination revealed edema of the right chest and right abdominal wall. A High-Resolution Computed Tomography (HRCT) scan showed right axillary lymphadenopathy with imbibition of the soft tissue compartment of the right chest wall. Laboratory investigation revealed increased myocytolysis indicators. While the patient was initially afebrile with normal vital signs, his clinical condition deteriorated while being evaluated with worsening Glasgow coma scale (12), decreasing blood pressure (90/50mmHg), tachypnea (22bpm), and metabolic acidosis. Blood tests showed normal levels of white blood cells, acute kidney injury, and an increase of the non-specific inflammation indices. Septic shock was suspected. Broad spectrum antibiotic therapy was started with a concomitant norepinephrine infusion due to his fluid resuscitation-resistant hypovolemia. In a few hours, the clinical condition of the patient deteriorated significantly and he died (22 hours after first ER admittance) of refractory septic shock and multiorgan failure.

The prosecutor ordered a forensic autopsy that was performed 48 hours later. The main external findings were multiple large reddish areas on the right hemithorax that spread down the right side and to the right gluteus with de-epithelialized areas. Histologic examination of the organs revealed widespread necrosis of the soft tissues and muscle fibers of the right hemithorax, buttocks, right flank, and pectoral region. The cause of death was certified as septic shock. Postmortem Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight (MALDI/TOF) mass spectrometry identified *Streptococcus pyogenes* as the cause of the NSTI. This finding was interesting because *Streptococcus pyogenes* is a typical cutaneous bacteria that rarely causes mono-microbial NSTI in the absence of a cutaneous lesion or in immunocompetent patients with no risk factors.

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#### NSTI, Spontaneous Infection, Streptococcus Pyogenes



### H60 Acute Colonic Pseudo-Obstruction (ACPO) (Ogilvie Syndrome) Leading to Respiratory Compromise and Death

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**Learning Overview:** The goal of this presentation is to educate attendees about a condition known as ACPO, or Ogilvie Syndrome, and how to differentiate it both clinically and at autopsy from true obstruction.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating an unusual case in which ACPO, or Ogilvie Syndrome, resulted in sudden death related to respiratory compromise.

In this case, a 19-year-old severely disabled man with Cerebral Palsy (CP) and autism experienced shortness of breath in association with a markedly distended abdomen and subsequently had an unwitnessed cardiac arrest. Records at his care facility reported the patient had experienced shortness of breath and diarrhea earlier in the evening prior to the arrest. He was transported to an Emergency Department (ED), but resuscitative efforts failed to revive him. A significantly distended abdomen that was notably hard on palpation was noted.

At autopsy, external examination revealed developmental deformities consistent with CP, a markedly distended abdomen, and rectal prolapse. A postmortem radiograph revealed massively distended bowels; this finding was confirmed on internal examination where the small and large intestines were distended to such an extent that the diaphragm was displaced significantly upward bilaterally, with associated compression of the lungs. Further analysis of the gastrointestinal tract failed to identify any signs of perforation or obstruction.

Sectioning of the compressed lungs revealed dark red-blue, moderately congested, compressed parenchyma. Microscopically, the lungs showed considerable compression with areas of collapsed parenchyma and areas of pulmonary edema. There were also focal areas of hyperexpanded alveoli and extravasated blood. Microscopic examination of the Gastrointestinal (GI) tract was unremarkable. Subsequent record review revealed that the patient had experienced similar episodes in the past (relieved via decompression in the ED), but as he was a relatively new resident at the care facility, staff were apparently unaware of this history. In addition, the patient had been receiving multiple medications with constipation as a known side effect, and his laxative medications had been withheld for several days, which potentially aggravated the problem. The cause of death was determined to be respiratory compromise secondary to diaphragmatic compression as a result of ACPO, with contributing underlying factors of CP and autism, including possible medication mismanagement.

The present case highlights the fact that severe intestinal obstruction can lead to significant diaphragmatic elevation with accompanying respiratory compromise and death. When evaluating a decedent for a potential intestinal obstruction at autopsy, it is important to consider a number of potential etiologies, including physical/structural obstruction from tumor, compression, adhesions, volvulus, or intussusception. When careful evaluation fails to reveal a physical/structural obstruction, consideration must be given to other conditions, including toxic megacolon, postoperative ileus, drug-induced obstruction, and ACPO.

#### ACPO, Intestinal Obstruction, Death



## H61 Intramyocardial Lipoma of the Right Atrium: Two Cases Diagnosed at Forensic Autopsy

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**Learning Overview:** After attending this presentation, attendees will be aware of the possible effects an intramyocardial lipoma may have on a patient and the associated risks.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing suspicion of the role of a lipomatous tumor in accelerating death in correlation to external factors.

Lipomatous tumors of the heart are rare, usually asymptomatic entities that typically present as incidental findings at autopsy. A few case reports describe presenting symptoms such as dizziness and shortness of breath; it is estimated that approximately 17% of all patients with cardiac tumors exhibit neurological symptoms.<sup>1</sup> Intramyocardial lipomas are most commonly found in the right atrium and the left ventricle.<sup>2</sup> Both atrial and ventricular lipomatous tumors are typically incidental findings on echocardiogram; Magnetic Resonance Imaging (MRI) can be used to better characterize the fat stranding, density, size, and location, while biopsies can be performed to rule out malignancy. Two unrelated decedents with right atrial intramyocardial lipomatous tumors were first identified at forensic autopsy.

A 70-year-old moderately obese man with a history of peripheral vascular disease, emphysema, and essential hypertension was pronounced dead in the emergency room following an altercation in his home. He had recently developed symptoms of irregular heartbeat and sweating episodes; thyroid function testing was planned. He had also developed forgetfulness and disordered thought processes. Autopsy corroborated the medical history, showed evidence of dementia, and documented an intramyocardial lipoma in the right atrium measuring 3.2 x 2.4 x 2.0cm. The lipoma was located in the upper margin of the fossa ovalis between the inferior vena cava and the superior vena cava, and displaced atrial muscle; it did not reach the crista terminalis. Death was attributed to external factors.

A 55-year-old ill-appearing woman, found dead in her bed, with a history of hypertension, was suspected to have died of a drug overdose; although no drug paraphernalia was found at the scene, she had an antecubital ecchymosis and a possible dermal puncture in each antecubital fossa. Her heart demonstrated significant concentric left ventricular hypertrophy, with four-chamber dilatation, and a 3 x 5cm right atrial intramyocardial encapsulated lipomatous tumor. The tumor extended from the area adjacent to the superior vena cava down to the auricle and into the anterior and right anterolateral atrial wall, sparing the septum.

**Discussion:** Primary cardiac tumors are rare, with an autopsy incidence of 0.001% to 0.03%; only 25% of them are found in the myocardium.<sup>2</sup> Intramyocardial lipomas may cause hemodynamic and electrical conduction changes, resulting in arrhythmias that may cause sudden death; approximately 0.0025% of sudden cardiac deaths are estimated to occur from primary cardiac tumors.<sup>3</sup> A few reports describe intramyocardial lipomas that were identified during life, after patients presented with shortness of breath, dizziness, and/or hypertension, which warranted an echocardiogram. All were successfully surgically resected; patients had a speedy recovery (approximately eight days in the hospital) and were asymptomatic/lipoma free for follow-ups recorded from one to four years post operation. Prompt diagnosis of primary cardiac tumors and surgical resection has often led to complete cures; surgery with a minimally invasive approach offers a low-risk procedure with an excellent long-term prognosis approaching 100%.<sup>2</sup>

In the current study, one decedent's symptoms of irregular heartbeat and sweating episodes were attributed to probable thyroid disease. Emphysema may have masked dyspnea, and dementia may have interfered with diagnosis of neurological symptoms. In the second case, a lipoma might have accelerated hemodynamic compromise under conditions of drug-related hypoxia. In both cases, it was unclear whether the lipoma played a contributing role in death through vulnerability to arrhythmia. Both of these cases support the literature favoring surgical excision of intracavitary or intramyocardial lipomatous tumors to decrease the risk for antiarrhythmic firing or sudden death; if such a tumor is encountered at forensic autopsy, consideration should be given to its possible role in the mechanism of death.

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### Lipomatous Tumor, Right Atrium Intramyocardial Lipoma, Forensic Autopsy

### H62 A Fatal Case of Chemical Peritonitis Caused by a Spontaneous Rupture of the Pancreatic Pseudocyst: A Forensic Approach

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**Learning Overview:** The goal of this presentation is to examine the clinical and histopathological aspects of a fatal case of chemical peritonitis due to spontaneous rupture of the pancreatic pseudocyst in a patient with pancreatic adenocarcinoma.

**Impact on the Forensic Science Community:** This presentation describes a patient with pancreatic adenocarcinoma presented with spontaneous rupture of the pancreatic pseudocyst. This coexisting condition is difficult to diagnose, and prognosis remains poor despite the advances in technology. This presentation will impact the forensic science community by describing the necessity for an accurate autopsy to preserve the surgical field and assess the cause of death in a malpractice claim.

A pancreatic pseudocyst is a localized fluid collection that is rich in pancreatic enzymes, such as amylase, and is enclosed by a wall of fibrous tissue that is not lined by epithelium. The lack of epithelial lining distinguishes pseudocysts from other cystic pancreatic lesions. Pseudocysts are linked with the pancreatic duct system, either via direct communication or indirectly via the pancreatic parenchyma.<sup>1</sup> Regardless of the etiology of the pseudocyst, the incidence is low, ranging from 1.6%–4.5% in adults.<sup>2</sup> Pseudocysts are most commonly detected after pancreatitis or trauma and are associated with alcoholism and gallbladder disease. Spontaneous perforation and fistulization of pancreatic pseudocysts occur in fewer than 3% of cases.<sup>2,3</sup> Spontaneous rupture of pancreatic pseudocysts and/or fistulization has been reported in the stomach, duodenum, biliary tract, renal collecting system, colon, bronchial tree, and peritoneal surface.<sup>3</sup> Chemical peritonitis is another possible pseudocyst complication that can arise due to inflammation of the peritoneum caused by rupture of an abdominal organ. Symptoms may include severe pain, swelling of the abdomen, fever, or weight loss. Complications may include shock and acute respiratory distress syndrome. Many potential causes exist, and the peritonitis may be classified as primary or secondary, local or diffuse, acute or chronic, or according to the causative agent.<sup>4</sup>

**Case Report:** A 37-year-old alcoholic male smoker was admitted to the hospital with a two-day history of fever, diarrhea, and recurrent vomiting. Laboratory tests showed an increase in pancreatic enzymes, anemia, thrombocytopenia, and hyperglycemia. He was admitted to the intensive care unit due to the worsening condition that same day, but ultimately died after two days. Due to relatives' claims of malpractice, the prosecutor's office ordered an autopsy. A complete autopsy was performed within four days. On internal examination, 400cc of brownish liquid was observed in the abdominal cavity. After the dissection of the peritoneal surface, a nodular pancreatic tail mass measuring 30mm in maximal dimension was appreciated. The cervical and thoracic organs were dissected via Gohn's technique (i.e., en bloc), and a Rokitansky modified technique was utilized for the gastrointestinal tract. After fixation, the 30mm pancreatic mass was further assessed and found to be connected with the pancreatic duct system. The peritoneal surface was sampled. Hematoxylin-Eosin (H&E) -stained sections of pancreatic parenchyma revealed a haphazard arrangement of glands, nuclear pleomorphism, incomplete glandular lumina, luminal necrosis, glands adjacent to muscular vessels, perineural invasion, lymphovascular invasion, and severe cytologic atypia (i.e., cytologic enlargement, hyperchromasia, loss of polarity, prominent nucleoli). Sections of the pancreatic mass showed a 1cm thick and calcified cyst wall, with a lack of epithelial lining. H&E-stained sections of the peritoneal surface showed an inflammatory reaction with associated neutrophils. Overall, the medical record data, complete autopsy, and macroscopic and microscopic pancreatic findings confirmed that the cause of death in this case was chemical peritonitis from spontaneous rupture of a pancreatic pseudocyst.

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#### Pancreatic Pseudocyst, Spontaneous Rupture, Pancreatic Adenocarcinoma

### H63 Idiopathic Liver Rupture: An Italian Case Report

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**Learning Overview:** After attending this presentation, attendees will understand that spontaneous bleeding due to a non-traumatic liver rupture is a rare but possible occurrence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that in cases of hemoperitoneum, spontaneous liver rupture should be considered, not only in patients with known predisposing factors.

Non-traumatic liver rupture is not uncommon in pregnant women with Hemolysis, Elevated Liver enzymes, and Low Platelet count (HELLP) syndrome and in association with some liver diseases, but very rarely occurs among healthy individuals.<sup>1</sup> It is usually diagnosed by imaging studies such as ultrasonogram or Computerized Tomogram (CT) or may be detected as an incidental finding in an emergency laparotomy. Very few cases of idiopathic liver rupture have been reported in literature. Reported here is the case of a patient with three visits to emergency health care services over a four-day span. Ultimately—although managed surgically—he died of a liver rupture without history of antecedent abdominal trauma or other clear etiology.

A 72-year-old man presented to the emergency department with abdominal pain. Physical examination elicited pain in the left hypochondrium and a positive Murphy sign. Laboratory tests revealed only a slight rise in white blood cells with neutrophilia, while abdominal ultrasonogram showed a “slightly thickened gallbladder wall.” He was discharged with instructions to return the following morning for a surgical evaluation. The following morning, he returned to the same hospital where the surgeon diagnosed him with acalculous cholecystitis. Two days later, he returned to the emergency room with fever (39°C) and abdominal pain. Laboratory studies showed the following: hemoglobin 12.3gm/dl, white cell count 10.600/cmm, platelet count 97.000/cmm. Liver function tests showed elevated bilirubin and enzymes.

The patient was admitted to the general surgery department where abdominal ultrasonogram raised the suspicion of a perihepatic fluid effusion. That night, the patient developed diarrhea. Early the following morning, he was found to be in shock with a blood pressure of 80/60mmHg. Laboratory studies showed a significant increase in liver function indices, severe anemia, and low hemoglobin (Hb 9g/dl at 4:15 a.m. → 5.90g/dl at 8:06 a.m.). CT revealed a large intraparenchymal and subcapsular hematoma with hemoperitoneum. At emergency laparotomy, there was blood in the peritoneal cavity while the right hepatic lobe showed a deep laceration between the 7<sup>th</sup> and 8<sup>th</sup> segments. Other solid viscera and bowel were unremarkable. Peritoneal washing and perihepatic packing and hemostasis were performed. Despite these measures, the patient died the next day. An autopsy was requested. On internal examination, the liver grossly demonstrated a rupture on the posterior hepatic surface (segments 7–8) and multiple hemorrhagic infiltrates (more marked in the right lobe sections.) Histologic sections showed extensive hepatic necrosis and hemorrhage, including large areas of coagulative necrosis of hepatocyte trabeculae with a central-midlobular extension.

In conclusion, this case provides many topics for thought. First, spontaneous intrahepatic hemorrhage and liver rupture usually occur in patients with predisposing factors, such as hepatocellular carcinoma or adenoma, HELLP syndrome, Ehlers Danlos disease, and graft-vs-host disease.<sup>2</sup> Second, spontaneous liver rupture should be considered in cases of atraumatic hemoperitoneum. Third, a high index of suspicion is required for early diagnosis and intervention to reduce morbidity and mortality in cases of liver rupture. In this case, spontaneous liver rupture and hemoperitoneum occurred in a patient without known risk factors, a very rare occurrence, making early diagnosis and treatment even more challenging.

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#### Liver, Rupture, Spontaneous



### H64 A Determination of the Efficacy of Two Processing Methods for Molar Teeth as a Source of DNA in Missing Persons and Unidentified Human Remains (UHR) Investigations

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**Learning Overview:** The goal of this presentation is to provide attendees with an empirical evaluation of two different processing methods for molar teeth as a source of DNA in missing persons and UHR casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the specific approach used to prepare a molar tooth for DNA extraction can impact DNA recovery and downstream Short Tandem Repeat (STR) typing results.

Bones and teeth are a valuable source of DNA in forensic, anthropological, and archaeological investigations. There are many scenarios in which bones/teeth may be the only viable sample type for DNA testing, including fires, explosions/bombings, natural disasters, war conflicts, airline crashes, homicides, and mass graves. Typically, compact weight-bearing long bones (such as the femur or tibia) and molar teeth are the preferred sample types for forensic genetic testing of skeletonized or highly decomposed remains. There currently is not a standardized protocol for processing molar teeth for DNA extraction. Some forensic laboratories pulverize the entire tooth, while others horizontally transect the tooth and drill into the inner cavity of the crown and roots to collect tooth powder. Pulverization of the entire tooth is a less time- and labor-intensive approach, and it requires less manual manipulation of the sample (an important consideration in contamination prevention). However, pulverization is a destructive process that completely consumes the sample, eliminating the possibility of re-testing and/or additional analyses that may be informative (e.g., amino acid racemization, isotopic analysis). In contrast, horizontal sectioning preserves the structural integrity of the tooth (which may be important for museum or other precious specimens), although it generates less powder for DNA extraction. In highly degraded remains, the latter approach may be prohibitive to recovery of sufficient DNA to make an identification or association.

Understanding the structural, molecular, and biochemical composition of teeth is an important consideration for forensic genetic testing. A molar tooth can be divided into two physical regions: the crown (the portion that visibly extends above the gumline) and roots (which are embedded within the alveolar bone of the maxilla and mandible). Additionally, teeth consist of four molecularly differentiated layers: enamel, cementum, dentin, and pulp. Enamel (the hardest substance in the human body) and its adjacent layer (cementum) provide a strong physical barrier that protects endogenous DNA from environmental insult. However, fractures or caries in enamel expose the inner layers of the tooth (and endogenous DNA) to damage, as well as to exogenous contamination. Dentin, which lines the inner pulp cavity, is targeted for DNA testing because it is rich in hydroxyapatite. Hydroxyapatite directly interacts (binds) with the backbone of the DNA molecule, purportedly protecting DNA from degradation. Often the pulp (soft tissue) within the crown of the tooth has decomposed by the time skeletal remains are recovered, leaving the rigid microstructure of the tooth as the only potential source of DNA. A principal rationale for using targeted drilling to process molar teeth lies in the protection afforded to the DNA molecule via direct interaction and binding to hydroxyapatite in the dentin layer.

This research specifically focused on comparing the effectiveness of two common mechanical processing strategies—complete pulverization vs. targeted drilling—for: (1) burned molar teeth, and (2) teeth that have been subjected to burial in acidic soil. These conditions reflect two of the most common forensic casework situations in which skeletonized remains are recovered. Effectiveness of each tooth-processing method was determined via comparison of total DNA recovery (ng), average signal per locus (peak heights, in Relative Fluorescence Units [RFUs]), and total number of alleles detected (i.e., completeness of Short Tandem Repeat [STR] profiles). Additionally, special considerations for targeted drilling techniques (and potential associated complications) will be discussed.

#### Unidentified Human Remains (UHR), Molar Teeth, DNA



### H65 The Vitality of Skin Lesions in Decomposed Corpses: A Morphological and Immunohistochemical Study

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**Learning Overview:** After attending this presentation, attendees will be better informed about a new combination of morphological and immunohistochemical methods useful in the evaluation of skin lesion vitality in decomposed corpses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by experimentally validating a comprehensive protocol applicable, even in a judicial context, in cases of decomposed corpses when traumatic death is suspected.

The present research investigates three different aspects of the issue of skin lesion vitality, including the correlation between morphologic and immunohistochemical data, the differentiation between hypostasis and vital blood infiltration, and the identification of a suitable immunohistochemical panel of red blood cells markers (i.e., hemoglobin, glycophorin A and  $\beta$ -spectrin). The evaluation of wound vitality has always been one of the most debated topics in forensic practice. Especially in decomposed bodies, determining if a skin's hemorrhagic infiltrate should be considered a vital tissue reaction or just a postmortem phenomenon is still a challenge for forensic pathologists. As is well described in literature, morphological and immunohistochemical methods have proven to be useful in the assessment of skin lesion vitality.

In this study, ten cases of traumatic immediate death with a postmortem interval of <72 hours were selected. To delete confounding factors, corpses with poor hypostasis or cutaneous putrefactive changes were not included. At forensic autopsies, two skin samples were taken from each case (i.e., one from a recent lesion and one from a hypostatic region). Each sample was experimentally kept in incubator at a temperature of 20°C to allow for experimental decomposition. Histologic specimens were obtained at defined time intervals (i.e., 0, 7, 15, and 30 days) from each sample. Each specimen was formalin-fixed, paraffin-embedded, then stained with Hematoxylin-Eosin (H&E) and Masson's trichromic, as well as with Immunohistochemical (IHC) stains for hemoglobin and glycophorin A and  $\beta$ -spectrin. As a negative control, undamaged and non-hypostatic skin was utilized and treated as described above. Finally, the samples were examined with an optical microscope by two independent observers.

Study results showed good correlation between morphologic and immunohistochemical data in the early period of putrefaction. After longer postmortem intervals, IHC proved to be more effective than H&E and Masson's trichromic for the detection of bleeding and for differentiating bleeding from hypostasis. The usage of more than one IHC marker as opposed to a singular marker improved the evaluation. Overall, such results suggest the usefulness of performing complete skin dissection during putrefied bodies' autopsy, and collecting—for histologic analysis—all lesions suspected to be vital. These samples should be microscopically analyzed using both classic histologic methods as well as multiple IHC markers.

#### Wound Vitality, Decomposition, Immunohistochemistry





### H66 Mendelian Simulation of Short Tandem Repeat (STR) Genotypes From Existing Sample Data to Create Standard Truth Pedigrees for Benchmarking New Familial Inference Methods

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**Learning Overview:** The goal of this presentation is to provide an overview of how to create simulated truth STR genotype data for benchmarking new pedigree inference methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information regarding creating simulated truth STR genotype data for benchmarking new pedigree inference methods. This work describes methods and software needed for simulating STR genotype benchmarking data that can assist in development, testing, and evaluation of a new familial searching methodology.

Current methods used in forensic DNA analysis evaluate the likelihood of a match based on STR length. Recently, there has been interest in extending the use of DNA databases beyond exact matching toward identifying potential familial relationships between individuals in a DNA database and/or with an unidentified person of interest whose DNA is found at a crime scene. When developing algorithms for identifying relationships, inferring pedigrees, and quantifying uncertainty, it is critical to have known ground truth data against which novel algorithms can be benchmarked. This data should include STR genotype profiles together with complete pedigree information against which inferences can be compared.

This study describes computational procedures developed for sampling individuals from an existing database and simulating offspring having STR genotypes following Mendelian rules of inheritance. In the absence of an existing database, publicly available allele frequency information can be used to simulate a founder population from which simulated offspring can be generated. By adjusting parameters, such as population size, Poisson rate of gravidity, endogamy, etc., population dynamics can be tailored to mimic those of an actual population of interest. The resulting simulated population will contain multigenerational pedigrees having individuals with STR genotypes where all individuals' relationships are known (e.g., parent-offspring, full sibling, half-sibling, and more distant second- and third-degree relationships). This methodology can be used to simulate pedigrees with STR data from any population at any number of loci where existing data are available, not just those where STR population allele frequencies are publicly available. The entire process is implemented in the R programming language, consisting of two modules. Module 1 includes procedures needed for ingesting, cleaning, and extracting STR genotypes from unrelated individuals in an STR genotype database. This module can be omitted or may need adapting, depending on the structure and quality of the input data. The second module comprises a method for simulating offspring and tracking relationships among initial founders and simulated offspring. Female individuals can be created from an all-male database by removing Y-chromosomal STR (Y-STR) genotypes. Mating events are created by randomly sampling two individuals from a pool of founders to create one or more children. Rates of half-sibling mating is also an adjustable parameter. The resulting simulated data can be used as a standard to assist development and conduct rigorous benchmarking of new methods for familial DNA searching, relationship inference, and quantifying uncertainty.

#### Bioinformatics, DNA, Simulation



### H67 An Investigation on Circular RNA (circRNA) Expression in Diabetic Cardiomyopathy (DCM) to Improve Understanding of Sudden Cardiac Death (SCD)

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the underlying relationship between DCM and SCD, as well as the present unveiled functions of circRNA in forensic applications and its possible role in DCM.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by revealing the potential role of circRNA in DCM when associated with SCD, thus laying the foundation for further study of the pathogenesis in DCM and promoting understanding of SCD.

SCD is a devastating outcome of a number of cardiovascular disorders, and determining SCD etiology/pathophysiology continues to be a ongoing challenge for forensic scientists. DCM, an severe cardiovascular complication of diabetes, has been proven to be strongly associated with SCD. Nevertheless, the pathogenesis of DCM remain to be fully elucidated. In recent years, a novel type of non-coding RNA known as circRNA was found to play a significant role in various diseases and showed great promise in forensic applications. Characterized by stability and tissue-specificity, circRNAs could not only serve as an ideal biomarker, but could also be of help in elucidating the mechanisms underlying various diseases.

In this study, the hearts of db/db mice were harvested and the circRNA expression of these hearts was analyzed using high-throughput RNA sequencing technology. Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR) analyses were used to validate the expression levels of circRNAs. Co-expression networks of circRNA-microRNA (miRNA) were constructed based on the correlation analysis between the selected circRNAs and their target miRNAs, and a further Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses for these circRNAs interacted miRNAs and their target genes were investigated. To excavate the key circRNAs in DCM, significant miRNAs that have been confirmed to be related to myocardial alterations were searched and the potential competing endogenous RNA (ceRNA) regulatory network was predicted. Finally, Internal Ribosome Entry Sites (IRES) of circRNA were predicted to detect the protein-coding potential of these circRNAs.

A total of 58 significant differentially expressed circRNAs were captured in the db/db sample. Six upregulated circRNAs and seven downregulated circRNAs were detected by RT-qPCR and analyzed through the GO and KEGG method. Ultimately, five key circRNAs (mmu\_circ\_0000652, mmu\_circ\_0000547, mmu\_circ\_0001058, mmu\_circ\_0000680, and mmu\_circ\_0001160) and a newly identified circRNA (novel\_circ\_0004285) were predicted to have potential binding sites with respective miRNAs that were firmly involved in myocardial injury. IRES predictions indicate that mmu\_circ\_0001160 and novel\_circ\_0004285 may have the potential to be translated into proteins.

Taken together, this analysis unveiled potential biomarkers and molecular mechanisms in DCM, which could promote the understanding of the pathogenesis of DCM. Moreover, further studies on the promising circRNA related to myocardial injury could be of help in exploring the underlying mechanisms of SCD.

#### CircRNA, Diabetic Cardiomyopathy, Sudden Cardiac Death



### H68 The Effects of Heat and Explosions on Forensic DNA Analyses

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**Learning Overview:** After attending this presentation, attendees will see the results of a four-year-long project that was completed in July 2019. This presentation includes specific temperature treatments that are required to produce partial DNA profiles from biological stains, the different effects on DNA that are caused by different types of explosions, and how the strength of such evidence (partial profiles) is affected. Attendees will also see how such challenging samples were analyzed through both the conventional capillary electrophoresis technique and massively parallel sequencing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing what may occur to biological evidence in cases that involve fire, explosions, or other sources of high heat. The samples were chosen to reflect realistic situations in which the identification of both victims or suspects is required. The community will also assess the uses and limitations of applying capillary electrophoresis-based methods or massively parallel sequencing to such a challenging type of casework.

This project explores the effects of high temperatures and explosions on DNA samples of forensic value. This project aims to determine the conditions under which biomaterials may degrade in accidental disasters, bombings, and/or crimes involving fire, and how degradation affects interpretation. The experiments were designed to reflect situations requiring victim and suspect identification, and thus involved testing various human samples, including blood and saliva stains. Some samples were heated under laboratory conditions, while others were attached to pipe bombs and detonated outdoors with the assistance of United States police bomb enforcement officers. A sensitive mitochondrial DNA multiplex system was devised and successfully used to detect DNA degradation prior to more costly analyses. Capillary electrophoresis-based Short Tandem Repeat (STR) typing and massively parallel sequencing were compared in terms of their performance on degraded DNA. Treatment at 180°C for 30 minutes was required to induce the first signs of DNA degradation in dried blood and saliva stains, reflected by reduced post-Polymerase Chain Reaction (PCR) DNA detection or drop-out of longer amplicons. There were no interpretable DNA products when heat treatment increased to 200°C. Similar degraded DNA effects were observed in 27% of stains placed on and within smokeless powder-charged pipe bombs, but no sign of degradation was observed with the more energetic C4 explosive, probably because of the shorter duration of heat exposure.

DNA degradation poses challenges to the interpretation of retrieved genetic data. These challenges were investigated both in real profiles from heat-treated samples and in simulated data. In addition, a real case of an unidentified male victim (the Blazing Car Murder, 1931) was analyzed. The combination of real and simulated data provided realistic scenarios, but also allowed control of parameters that affect evidential strength, such as population size and diversity, through lowering of the likelihood ratio and increasing the number of random matches in a database.

#### DNA Degradation, Partial Profiles, Extreme Conditions

## H69 Creating a Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR) -Based Method for Studying Temporal DNA Degradation in Waterlogged Bone

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**Learning Overview:** After attending this presentation, attendees will better understand the influences of time and temperature, type of bone, extraction method, and source of freshwater on DNA retrieval, variation in quantity, and variation of degradation in waterlogged porcine skeletal remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information concerning the ideal time interval in which DNA can be successfully obtained and amplified from submerged bone samples. Additionally, attendees will understand how extraction methods and skeletal element selection impact DNA extraction and amplification. Ultimately, laboratories will be able to evaluate whether submerged bone samples will provide viable DNA, thus saving time, labor, and money.

Human activities are often centered around the presence of water; thus, it is not surprising that there are many water-related human deaths, including those from mass disasters, murder, and/or clandestine body disposal. Scavenger activity, Accumulated Degree Days (ADD), and other aquatic variables may affect DNA retrieval from waterlogged bone. Organic and inorganic material in bone have an increased vulnerability, as water causes bone dissolution. This process can destroy the DNA contained in the osteocytes.<sup>1</sup> Calcium and collagen in bone can inhibit the PCR necessary to produce a Short Tandem Repeat (STR) profile; the current solution is a time-consuming organic extraction, reported to produce a 99% recovery rate.<sup>2</sup> While there are examples of research on DNA degradation in terrestrial bone over time, there has been little work done on submerged bone samples.<sup>3</sup> Although case studies are not controlled experiments, they are informative for research on human samples. One case report concerns skeletal and soft tissue remains of a murdered child who had been submerged in water for up to three years. DNA retained in the bone was still extractable DNA in this case.<sup>4</sup>

This research project was based on a long-term, controlled experiment that offers potential answers to the problem of DNA retrieval in waterlogged bone. The research also provides a shorter solid-phase protocol and a way to estimate over what length of time/temperature (in ADD) DNA may be extracted from skeletal remains, thus allowing a crime lab to determine if effort should be applied.

Fresh rib and scapulae from pigs (*Sus scrofa*) were submerged at Henleys Lake (November 2016–November 2017) and in the James River (November 2017–November 2018), with water temperature and quality measured every 500 ADD, using a 0°C as a base temperature for ADD calculation. The total sample size began with 200 ribs and 200 scapulae, though 46 samples were lost over time due to varying factors. In the lab, they were cut into pieces, placed into a mortar with liquid nitrogen, and ground into powder. Some of the bone powder was used in the ChargeSwitch® gDNA Plant Kit, following the Invitrogen™ CST Protocol for Extracting gDNA from Bone Samples (2009). The final elution volume was 100ul. The phenol-chloroform organic DNA extraction method followed Iyavoo et al., with a final elution volume of 150ul.<sup>5</sup> All samples were stored at -20°C until further analysis. These samples were amplified using PCR, and then subject to gel electrophoresis to determine the DNA quality. Those that failed to amplify (21.18% Chargeswitch, 22.32% organic) were all successfully cleaned up using the DNeasy® PowerClean Pro Clean Up kit. The cleaned samples were run through PCR and another gel was run to confirm quality. A TaqMan® qPCR approach was utilized to determine quality and quantity of extracted DNA. Two primer pair sets were chosen to amplify a short (62bp) and a large (147bp) fragment of porcine DNA extracted from submerged bone samples. For RT-qPCR, plates were run using the ABI® 7500 Real Time PCR Machine with analysis using SDS v1.x software. The statistics were run using fixed modeling on RStudio.

An Analysis of Variance (ANOVA) of the preliminary data indicates that extraction method ( $p=1.954e-05$ ) and bone type ( $p=.04104$ ) have a statistically significant ( $\alpha=.05$ ) effect on DNA quantity, while the location of the freshwater body did not. The overall results suggest that: (1) the simpler, time-saving ChargeSwitch® method is more effective than the current protocol of organic extraction; and (2) that one bone is better for extraction than another. Results for the DNA degradation index over the submersion interval are forthcoming.

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### Skeletal Remains, RT-qPCR, DNA Analysis

### H70 Forensic DNA Phenotyping (FDP): A Prediction of Human Externally Visible Traits in Missing Person Identification

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**Learning Overview:** The goal of this presentation is to inform attendees of a new “DNA intelligence” tool that is approaching forensic biology, due to its ability to predict Externally Visible Characteristics (EVCs) from biological material, such as that found at crime scenes, in disaster victim identification, or in cases of missing person identification. EVC prediction from forensic samples, or from body parts, is expected to help concentrate police investigations toward finding unknown individuals at times when conventional DNA profiling fails to provide informative leads.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by attempting to prove and underline the utility of FDP in missing person identification cases. Due to the lack of data in literature, this work involved the application of the HIRISplex system for eye, hair, and skin color prediction to 20 Italian missing person identification cases previously studied. In order to evaluate the accuracy of the multiplex tested, 20 cases for which victim pictures or portraits were available were chosen.

Predicting EVCs using informative DNA molecular markers has started to become a rapidly developing area in FDP. The most relevant forensic cases for DNA-based EVCs prediction would be those in which the evidence DNA is useful for describing a person’s physical appearance from skeletal remains. This study evaluates the HIRISplex DNA test multiplex for the simultaneous prediction of eye, hair, and skin color in 20 Italian skeletal remains from 20 different missing person identification cases in which identification was previously performed using the conventional Short Tandem Repeat (STR) -based method. In all 20 cases, victim pictures or portraits were utilized as a reference in order to test the reliability and accuracy of the DNA-based EVCs prediction.

The DNA-based system used consists of a SnapShot® multiplex assay targeting a total of 41 Single Nucleotide Polymorphisms (SNPs) involved in skin, eye, and hair color prediction<sup>1</sup>. A total of 20 different bone samples (14 femurs and 6 tibias) were evaluated and processed. Bone samples were cleaned chemically, using diluted bleach, and irradiated with Ultraviolet (UV) light for 30 minutes prior to grinding into a fine powder. Genomic DNA was obtained from 0.5g of bone powder using the QIAamp® DNA Investigator Kit. The DNA extracts were quantified using the Investigator QuantiPlex™ HYres Kit. The reactions were carried out in a Rotor-Gene® 5-plex System according to the manufacturer’s instructions. Polymerase Chain Reaction (PCR) amplification of all 17 SNPs was performed in a single multiplex PCR assay, in a final volume of 10µl, as described by Chaitanya et al.<sup>1</sup> The amplified PCR products were purified with Thermo Scientific® ExoI and incubated at 37°C for 45min and 80°C for 15min. Single Base Extension (SBE) was carried out for all PCR products simultaneously in a single multiplex reaction using 2µl of the purified PCR products. Finally, the purified SBE products were analyzed on the ABI® 310 HID Genetic Analyzer. Gene Mapper® ID-X v1.0 software program was used for the allele calling and analysis of the results. Prediction of eye, hair, and skin color was performed using a web interface at <https://hirisplex.erasmusmc.nl/> allowing the retrieval of individual prediction probabilities for three eye color, four hair color, and now five skin color categories from HIRISplex genotype input data of 41 SNPs. Each victim picture’s iris, hair, and skin color were subjectively and objectively determined for all 20 skeletal remains analyzed. For the subjective determination, examiners classified victims’ pictures in the same color category (i.e., iris, hair, and skin color) for 84% of the documents examined.

Study results showed overall prediction accuracies of 91.6%, 90.4%, and 91.2%, respectively, for iris, hair, and skin color at the 0.7 threshold. The sample set had only two inconclusive results as compared to the IrisPlex database. This is likely due to the fact that these samples showed an intermediate eye and hair color. In conclusion, these results demonstrate the robustness and reliability of the HIRISplex genotyping system. Utilization should be encouraged for the prediction of eye, hair, and skin traits from DNA in missing person cases and in outside anthropological applications to determine eye, hair, and skin color of deceased persons via analyzing skeletal remains or body parts.

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#### Forensic DNA Phenotyping, Predictive DNA Analysis, IrisPlex



## H71 The Detection of Rat Decomposition Products in a Plywood Platform Following Specimen Removal

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of cadaveric Volatile Organic Compounds (VOCs) and changing decomposition odor profiles across animal models as well as novel methods and techniques for sampling decomposition products in plywood matrices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the Decompositional Odor Analysis Database for a previously unstudied model organism, specifically the Sprague-Dawley rat in the advanced stages of decomposition.<sup>1</sup>

Twelve rats were placed in cages on separate plywood platforms and left to decompose outdoors for 222 days through the summer and winter months in Pennsylvania. Following the removal of the advanced-stage decomposition rats from a plywood platform, a modified arson Gas Chromatography/Mass Spectrometry (GC/MS) method was used to test for the presence of decomposition products (VOCs and Volatile Fatty Acids [VFAs]) in the wood of the cage. The goal of this study was to investigate the unique odor profiles of rats and potentially contribute to the growing database of decomposition odor profiles important for entomofaunal succession and cadaver dog training.<sup>2,3</sup>

Expanding upon research that looks to the soil of the Cadaver Decomposition Island (CDI) and the indoor surfaces upon which human decomposition products (microbial macromolecule degradation and the leaching of waste products from the body cavity) may be deposited, this project acted as a pilot study to look at how rat decomposition products were absorbed and retained by a wood platform in an outdoor environment.<sup>4</sup> Arson cans, activated charcoal extraction, and GC/MS were used to determine the presence or absence of cadaveric VOCs, VFAs, and amino acid metabolic products commonly cited in the literature in the wood platform.<sup>3,5</sup>

Numerous studies in multiple countries have been conducted to work toward a comprehensive understanding of the VOCs evolved from decaying human and animal remains as a function of time and environmental conditions, leading to the development of the Decompositional Odor Analysis Database.<sup>1</sup> This database can assist forensic investigators in determining which compounds to look for in cases where there is suspicion that a body has been moved postmortem or when authorities are looking for clandestine graves. However, while there seems to be great interest in this area, many researchers using a variety of different methods and animal models have found it difficult to obtain reproducible decomposition odor profiles that are of any use in postmortem interval estimations or standardized Human Remains Detection (HRD) canine training procedures.<sup>6</sup> While there is extensive research pertaining to VOCs in the soil and air around different animal models for decomposition, it does not appear that these volatile decomposition products have been studied extensively in rat models or from a wood matrix. Therefore, this research sought to determine if decomposition products could be detected from a plywood matrix using a modified arson method, and to use this method to identify the compounds present in the field samples qualitatively following rat removal.

The rats were in the advanced stages of decomposition at the time of removal from the plywood platform, so it was hypothesized that only the higher molecular weight compounds would be detected in the field samples. Few well-known decomposition products were recovered, such as 2-ethyl-1-hexanol, while most of the recovered compounds were indicative of the local entomofaunal activity and the presence of several plant species near the experimental set-up.<sup>7</sup> The modified arson method used shows potential for recovering a variety of compounds, such as cyclic amines and sesquiterpenes, from field samples, but future work is needed to optimize and validate this method.

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### Taphonomy, Decomposition, Volatile Organic Compounds (VOCs)



### H72 Aquatic Decomposition of Vertebrate Remains: An Experimental Test for a Cold Case Investigation

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**Learning Overview:** The goal of this presentation is to provide attendees with the ability to better understand how conditions in aquatic environments impact the decomposition of vertebrate remains and how such information can be informative to death investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on how insects and microbes colonize and change on remains in aquatic environments and how decomposition progresses under environmental conditions in a way that informed a cold case death investigation.

Decomposition of remains in aquatic environments remains an understudied area of forensics, especially under conditions with direct relevance to death investigations. The objectives of this study were to determine how terrestrial and aquatic invertebrates and bacteria colonize and change on vertebrate remains over the course of decomposition and compare those findings to a cold case in a similar habitat that occurred in 2005 (referred to as the Aquatic Case [AC]).

At a similar time of year to the AC (July-August), swine carcasses ( $N=5$ , approx. 45kg) were euthanized and transported to the study location before being dressed in clothing to resemble the apparel found on the AC. The carcasses were introduced to the pond and evaluated for invertebrate activity at three-day intervals. In addition to evaluating the carcass for invertebrate activity and visual changes, cotton swabs were used to collect DNA samples from several different regions on the carcasses. DNA present on these swabs was extracted using a commercially available kit and quantified fluorometrically. Targeted amplicon sequencing was performed to amplify the 16S ribosomal RNA (rRNA) region (V4) using an Illumina<sup>®</sup> MiSeq<sup>®</sup> platform (2 x 250 base pair, paired ends). Resulting sequences were filtered and taxonomically assigned using QIIME<sup>™</sup> 2 and the SILVA database before open source statistical tools, including machine learning algorithms, were used to identify bacterial indicators of decomposition stage.

During the experiment, there was not a significant difference in air temperatures at the experimental pond compared to those recorded during the 21 days when the decedent of the AC was missing in 2005. Within three days of carcass placement, there was considerable bloating and large numbers of larval and adult blow flies (Diptera: Calliphoridae) present on all experimental carcasses as well as multiple taxa of aquatic invertebrates. When comparing the experimental results to the lack of any insect evidence reported in the AC autopsy and death investigation reports, the findings suggested that the AC body had a low probability of being at or near the surface for several days; these findings matched autopsy photos and medical examiner notes indicating only moderate decomposition. While the experimental results suggested extensive colonization of a carcass at the surface by both terrestrial and aquatic invertebrates would occur quickly, the potential remains that the AC body could have sunk and remained neutrally buoyant for 20 days at a depth where decomposition was slowed. Significant changes in the bacterial communities were found across decomposition with distinct bacterial communities present at different decomposition stages. Several diversity metrics including Faith's Phylogenetic Diversity (PD) and observed richness significantly differed among decomposition stage, with freshly submerged carcasses showing the highest bacterial diversity. Several bacterial genera, predominately from *Proteobacteria* and *Firmicutes*, were identified by as indicator taxa for decomposition stage by machine learning models. Future aquatic death investigations could take advantage of taking bacterial swabs that could be used as a "microbial clock" of decomposition in the aquatic habitat using the methods tested in this experiment.

In conclusion, this research highlights that the decomposition of vertebrate remains in aquatic environments is a complex process mediated by both biotic and abiotic factors and that both microbes and invertebrates change over the course of decomposition and have the potential to be used as forensic indicators.

#### Aquatic Decomposition, Microbiome, Cold Case

## H73 The Alterations of HMGB and Troponin in Postmortem Interval (PMI): The First Experimental Study on Humans and a Review of Literature

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**Learning Overview:** After attending this presentation, attendees will understand the role of High-Mobility Group Box-1 (HMGB) and troponin in PMI estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the quantitative alterations of these proteins in the PMI on humans.

Time of death represents a challenge in forensic research. Multiple extrinsic factors may make it challenging to estimate PMI. Several authors have suggested employing new scientific approaches, such as proteomics, to help improve estimation accuracy. In the forensic literature, numerous studies have emerged describing the changes proteins within tissues or biological fluids undergo with advancing PMI. However, most of these studies were carried out on animals or on corpses found after several hours, without knowing the exact hour of death. In fact, before the collection of the samples, the corpse has already been exposed to the action of extrinsic factors that are often unknown. This presentation proposes the results of an Enzyme-Linked Immuno-Sorbent Assay (ELISA) analysis carried out on human plasma taken at the exact time of death. The markers examined were HMGB and Troponin T (TnT). This is the first experimental study on human samples performed at “zero time.”

The experimental model was created at the Institute of Legal Medicine of the University Magna Graecia of Catanzaro. The model is based on taking peripheral blood samples from patients who died at the intensive care unit. The study was approved by the Ethics Committee. The informed consent was signed by family members before the death of the patient. The corpses were exposed to constant room temperature. The samples were taken at seven predefined time intervals, starting from the exact moment of death up to the next two hours. The samples were immediately centrifuged and stored at -80°C. Subsequently, the samples were analyzed by ELISA 96-wells in order to examine the quantitative changes of the HMGB and TnT markers. The kits used were MyBioSource® with human target. The following procedure was conducted: (1) add 100µL standard or sample to each well. Incubate for 90min–2hrs at 37°C; (2) remove the liquid. Add 100µL Biotinylated Detection Ab. Incubate for one hour at 37°C; (3) aspirate and wash three times; (4) add 100µL Horseradish Peroxidase (HRP) conjugate. Incubate for 30min at 37°C; (5) aspirate and wash five times; (6) add 90µL substrate reagent. Incubate for 15–30min at 37°C; (7) add 50µL stop solution. Read at 450nm immediately; and (8) the results were compared with the data published in literature.

Twenty-four cases were collected and divided into two main groups by age (cut-off 69.6 years), sex, and cause of death. Of these, ten cases were selected in proportional numbers from each group in order to guarantee the homogeneity of the sample and reduce the role of intrinsic potential variables. The patients were both male and female, aged between 56 and 86 years, and died from cardiogenic shock (seven cases), respiratory failure (two cases), and septic shock (one case).

This study showed a variability of HMGB-1 over time, with increasing levels of the marker at 2h compared to “zero time” (seven cases/ten) regardless of the cause of death. In the other three cases, an oscillation of the marker was observed with substantial stability. These results appear in agreement with the published literature regarding the potential increase in HMGB-1 expression levels as a function of PMI.<sup>1,2</sup> TnT revealed levels of variable expression within a wider range. Troponin levels were higher at 2h in seven cases out of ten. The increase was progressive and linear in cases of myocardial ischemia. A reduction in expression at 2h was observed in three cases of cardiogenic shock with systemic failure, pulmonary fibrosis, and septic shock. These results, also in agreement with the literature data, suggest that the marker levels strongly depend on the cause of death.<sup>3,4</sup> Being a marker of muscle damage, its expression would therefore be expected to be greater in cases of cardiac death. Although the analyzed sample is small, the proposed model is the first to perform proteomic investigations on human biological samples from the exact moment of death without exposing the corpse to temperature variations or other extrinsic factors.

In summary, this operating model is intended to identify the possible role of peripheral blood protein biomarkers procured at the precise time of death in the estimation of PMI, verify and evaluate the variation of the proteomic profile of markers already published in the literature, and improve analysis of the so-called “early PMI” for forensic purposes.

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### Forensic Sciences, Proteomics, Time of Death

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### H74 Fetoneonatal Deaths—The Role of Forensic Investigations and Differential Diagnosis for Solving Complicated Forensic Cases: A Rare Case of Infanticide

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**Learning Overview:** After attending this presentation, attendees will be able to describe the role of differential diagnosis in cases of infanticide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of a standard forensic protocol for this diagnosis.

The term “stillbirth” refers to a fetus born dead and with a gestational age of at least 28 weeks.<sup>1</sup> In Western countries, fetal-neonatal mortality is 3 deaths per 1,000 births, while in underdeveloped countries it is ten times higher. The causes of death include perinatal asphyxia, infections during pregnancy, maternal disorders, congenital anomalies, or abnormal fetal growth.<sup>2,3</sup> Death is not always the result of pre-natal/perinatal complications but also of crimes, such as infanticide. Infanticide is the murder of an unwanted newborn or a baby within the first year of life. Established parental risk factors for infanticide include a history of child abuse, domestic violence, psychiatric disorders, and economic hardship.<sup>4,5</sup> In such cases, the role of the forensic pathologist is to establish whether the fetus was born alive and determine the cause of death. There are no published standardized forensic procedures for differentiating between infanticide and fetal-neonatal death from other diseases. A forensic protocol for cases of suspected infanticide is proposed and explored via presentation of an educational case example.

A woman presented to the hospital with hemorrhage and showing signs of a recent parturition. The physicians asked about the birth and health of the neonate. She confessed that she had given birth to a dead fetus. In her house, the fetus was found dead. An autopsy was performed. External examination showed a term fetus with no congenital anomalies. There was cyanosis of the nail beds, cutaneous pallor, subconjunctival hemorrhage, and traces of meconium. The umbilical cord, measuring 68cm, was around the neck. It was ischemic and appeared to have been cut. The placenta showed no anomalies. Internal examination showed some lung petechiae; a hydrostatic lung test was positive. Histologic sections showed the alveoli were expanded and functioning when he was born, with signs of acute pulmonary emphysema and rupture of the alveolar septae. A forensic mother’s examination was performed excluding uterine or vaginal diseases. The investigations showed that the birth had occurred regularly. Death was due to bleeding because the umbilical cord was not clamped, with asphyxia also.

Infanticide is a worldwide issue.<sup>6</sup> In literature, infanticide is related to maternal psychiatric disorders. Infanticide sometimes occurs in the absence of support for the mother.<sup>4</sup> The case reported is emblematic. The mother gave birth alone at home and cut the umbilical cord, without any health or family care. Asphyxia is the most common cause of death. In such cases, establishing whether the fetus suffered from perinatal complications (e.g., an umbilical cord looped around the neck) is critical. Features supportive of asphyxia include a cord that is very long and fetal petechiae. The forensic examinations in the reported case showed that the fetus had breathed and the cord was not clamped. Death was due to both hemorrhage and asphyxia and—had routine medical care been provided—it is suspected the newborn would have lived. This case shows that the diagnosis of infanticide is challenging, and there can be various modes of death. A standardized forensic protocol can assist in detecting infanticide cases and prevent them from remaining unsolved. A recommended investigative protocol includes the following: (1) evaluate anthropometric data and signs of fetal maturity; (2) perform full fetal autopsy, including histopathology with microscopic analysis of the lungs; (3) measure the length of the umbilical cord and evaluate for placental abnormalities; (4) perform a physical examination of the mother to exclude illegal abortion; (5) compare the data with the mother’s witness reports; and (6) assess the mother’s psychiatric comorbidities for imputability.<sup>7</sup>

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#### Forensic Sciences, Infanticide, Asphyxia

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### H75 Bleach Decontamination in the Forensic Laboratory and at the Crime Scene: Investigating the Efficacy of DNA Damage in Native Versus Naked Templates

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**Learning Overview:** The goal of this presentation is to educate attendees about differences in the efficacy of bleach in generating damage to native and naked DNA templates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that amplifiable DNA can often still be recovered from human blood that has been exposed to bleach, especially if the DNA is still encompassed in its native tissue upon initial exposure.

In forensic casework, there are three major factors that significantly impact successful recovery of a DNA profile from evidence, including low-quality (degraded) DNA, low-quantity DNA, and the presence of endogenous or environmental inhibitors. The latter two factors have largely been mitigated by recent advances in instrumentation, “increased sensitivity” methods, and improvements in DNA extraction techniques. However, DNA damage/degradation is inherent in an evidentiary sample when it arrives in the laboratory. The degree and spectrum of DNA damage present depends on the environment to which it was exposed and the length of exposure time. In the natural environment, ultraviolet light, acidity, heat, and humidity all contribute to various forms of damage in the molecular structure of DNA. In addition to environmental insult, chemicals can be used to damage DNA. Bleach is used intentionally by criminals to clean up crime scenes and destroy DNA evidence.

Bleach (sodium hypochlorite [NaOCl]) degrades DNA through oxidative damage and production of chlorinated base products. Knowledge of this damaging effect of bleach on DNA is the basis for its use in forensic laboratories to clean workbenches and prevent cross-contamination of samples between cases. Although decontamination procedures in a forensic laboratory setting are carried out with dilute (10%) bleach, criminals are likely to use much higher concentrations (100%) in attempts to destroy DNA evidence. A previous study demonstrated that bleach has a decreased effect on native DNA that is still encompassed within a body fluid (compared to naked DNA that has already been extracted).<sup>1</sup> Completion of the clotting mechanism appeared to interfere with bleach’s ability to cause substantial DNA damage. This decreased effect of bleach on native templates may be explained in part by understanding the physical packaging of DNA, as it exists within human cells or body fluids. Nuclear DNA is not a “naked” molecule. In its native conformation, DNA is a supercoiled structure that is highly packaged into chromatin and is associated with a variety of other molecules, such as histone proteins, residual proteins, phosphoproteins, RNA species, and lipids. Hence, the manner or degree in which damage occurs to DNA in its native complexed form is likely quite different than in its “naked” counterpart. Native DNA may be afforded some protection from damage because it is surrounded by a cellular milieu of proteins, lipids, carbohydrates, and other nucleic acids (RNA).

This research expanded on a previous study, with an increased sample size and expanded data set. Whole human blood (native DNA) and extracted (naked) DNA were immersed in 10% and 100% bleach solutions for one hour. Ten times the volume of bleach was used for the damaging experiments on whole blood or naked DNA, respectively (10:1 bleach: blood/DNA). DNA extractions were performed with the QIAamp® DNA Investigator Kit; recovered DNA was quantified using the Quantifier™ Human DNA quantification kit and a 7500 Real-time Polymerase Chain Reaction (PCR) system. Results were consistent with the previous study. Sufficient DNA was recovered for Short Tandem Repeat (STR) typing, for both native and naked DNA templates and after exposure to both 10% and 100% bleach solutions (with higher DNA recovery from native templates and the lower percentage bleach concentration). These findings have value because they indicate that current decontamination methods using bleach in the laboratory may not be as effective as perceived (at least for DNA complexed with other materials). Decontamination of laboratory workbenches may actually partially be due to physical removal of DNA from a surface (“wiping away”) as opposed to solely chemical destruction. Additionally, it is often assumed that if a criminal has cleaned a crime scene with bleach, any underlying DNA evidence has been destroyed (which may prevent investigators from swabbing the area and submitting samples to laboratories for DNA analysis). This study demonstrates that evidentiary items presumably exposed to bleach still should be collected and submitted for DNA testing.

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#### Degraded DNA, Oxidative Damage, Bleach Decontamination



### H76 Vertebrate Scavenging Behavior and the Decay Rate of Buried Carcasses

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**Learning Overview:** After attending this presentation, attendees will better understand the role of animal scavenging during decomposition and its implications for forensic investigation and determination of postmortem interval.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an understanding of the impact of wildlife on carcasses in protected (burial) and unprotected (surface) outdoor environments and the timing of decay.

A 46-year-old woman was last seen alive in May of 2011. Eight months after her disappearance, her remains were found partially buried in a forested area in the inner middle region of Italy. The right femur, still attached to the body, was found above the ground surface. The remainder of the right lower limb was disarticulated and missing, while the remainder of the body was buried in a shallow grave a few centimeters deep. No other relevant disarticulation was observed. The head and the limbs were skeletonized while the rest of the body was well preserved and mummified. Insects on the body were identified as larvae of Syrphidae and Stratiomyidae. No Diptera of the first wave of colonization were recovered. According to these findings, the time of death was undetermined but consistent with a Postmortem Interval (PMI) of several months of burial. At autopsy, the cause of death was determined to be asphyxia due to strangulation. The victim's boyfriend was indicted for the crime. He provided an alibi for the time of death and claimed to be innocent. He recruited an expert who determined the PMI to be only a couple of months; the basis for this assertion was that a body buried in a forest area for longer would presumably have been scavenged by carnivores (e.g., boars, wolves, vultures), and thus the well-preserved state of the body would not be consistent with a longer PMI, such as eight months.

Can animal scavenging assist in estimation of the PMI? This is one of the most common questions asked of forensic experts since previous published studies have revealed a correspondence between stages of disarticulation and the PMI. A multidisciplinary team with forensic veterinary expertise was requested to provide a reliable answer. A field experiment using animal models was set up and performed in winter in a forested area. The goal was to determine the habits of scavengers when decomposing tissues are available in shallow graves or when exposed on the surface. Five carcasses were buried (two boars, one fox, one rabbit, and one hare). One fox carcass was exposed on the ground surface. Motion-sensitive cameras were placed in close proximity to the burial sites in order to check daily animal activity. Site visits were conducted every three days. The results showed that only the rabbit and the hare were scavenged: the first one a few hours after burial and the second one within seven days. The other four carcasses, including the fox on the surface, were not scavenged by wildlife. According to videotape recording, boars and wolves visited the experimental sites just around the carcasses, but without scavenging the remains.

These results confirm that: (1) burial can slow decomposition and influence the time required for insects to reach the remains; and (2) the scavenging of remains is not an event that necessarily occurs, even when there are decaying tissues exposed on the surface. Scavenging can be affected by several factors depending on hunger of the scavenger itself, intra- and inter-species competition, availability of other and more favorite food supplies, and the repellent effect of decompositional odors or invertebrate activity. Carnivores are unlikely to scavenge a corpse if there is a high concentration of insects until arthropod activity is over or decreased significantly. Vertebrate scavenging behavior is more common on fresh bodies, within the first hours after death, or in skeletonized corpses. The reasons for the low interest in putrefied carcasses can depend on: (1) access to the body; (2) the effort required by the scavenging; (3) freshness of soft tissue; and (4) the scavenger's food habits and diffidence toward new or different resources.

Finally, the absence of insect activity was the most important factor delaying decomposition, with only a very few signs of animal scavenging at the disarticulated lower limb. Overall, this field experiment highlights the importance of having knowledge of the type and behavior of local fauna as shallow graves can sometimes prevent not only insect activity but also scavenging by wildlife, thus explaining a slower rate of decomposition.

#### Forensic Taphonomy, Animal Scavenging, Buried Bodies

## H77 A Postmortem Interval (PMI) Estimation Based on Eukaryotic Community Associated With Soil Under Decomposing Porcine Remains

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**Learning Overview:** After attending this presentation, attendees will understand how eukaryotic community associated with soil under carrion can be utilized for PMI estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping to increase the accuracy and reliability of current statistical models for PMI estimation and by stressing future research on alternate techniques for PMI estimation with necrobiome sequencing.

Recently, there has been an increased interest in the necrobiome in soils associated with decomposing carcasses. Recent studies have shown bacteria associated with soils under a carcass change significantly both temporally and spatially.<sup>1</sup> However, limited information exists on temporal and spatial changes in eukaryotic community structure associated with soil under decomposing remains. To pursue this technique accurately, more replication in field conditions are needed, as well as a long-term study using a model organism similar to humans. This study was designed to fill in these gaps and expand on previous studies to improve PMI estimation techniques. The main aim of this study was to determine the eukaryotic community structure associated with soil under relatively well-replicated porcine remains for PMI estimation and determination of cadaver decomposition sites. To accomplish this goal, soil samples were collected at 0m (beneath the corpse) and 3m away from porcine remains ( $N=6$ ). The sample collections started at T0 (Day 0, before porcine remains were placed) and continued daily until Day 3. Samples were then collected on Day 5, then collected weekly until Day 60 (two months, or 1,703 Accumulated Degree Days [ADD]). DNA was extracted from soil samples using DNeasy® PowerSoil Kit following the manufacturer's protocol. The variable region V9 of 18S recombinant DNA (rDNA) was amplified on an Applied Biosystems® Veriti® 96-well thermal cycler utilizing the primers and protocol referred to by the Earth Microbiome Project.<sup>2</sup> The 96-well Polymerase Chain Reaction (PCR) plate included a negative (nuclease free water, Promega® Corporation) control sample. Following amplification, all PCR products were visualized on a 1.8% agarose gel to ensure successful PCR amplification and that the PCR products are of expected size. All PCR products were cleaned using Agencourt® AMPure® XP kit using the manufacturer's protocol. Purified amplicons from each sample were pooled in equimolar concentrations for 18S rDNA dual-index MiSeq® sequencing as described by Kozich et al. on MiSeq® FGx sequencing platform.<sup>3</sup> Sequence data will be analyzed using mothur v1.39.5.<sup>4</sup>

PCR amplification of 18S rDNA yielded two products (one with the expected size and the other with a much smaller size) for many soil samples. The band that had the expected size was gel extracted, cleaned, and used for sequencing purpose. This eukaryotic succession data, or the combination of the eukaryotic and bacterial succession data, will help in reducing errors associated with current statistical models for PMI estimation.

In conclusion, attendees will become aware of the current and emerging techniques used for PMI estimation. The results from this research can be applied to forensic investigations and offer additional detail at crime scenes. Ultimately, this study will aid in expanding the amount of information that can be utilized from evidence in a forensic setting based on necrobiome sequencing.

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### Postmortem Interval, Necrobiome, 18S rDNA

### H78 Atrioesophageal Fistulas (AEF) Caused by Percutaneous and Surgical Radiofrequency Ablation for Atrial Fibrillation (AF)

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**Learning Overview:** The goal of this presentation is to show the development of atrioesophageal after percutaneous and surgical radiofrequency ablation for chronic AF.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting that in patients with neurological clinical signs with a positive history of radiofrequency ablation (percutaneous or surgical) of AF, it is always desirable to consider the risk of an Atriobronchial Fistula (ABF) or AEF.

Atriobronchial Fistulas (ABF) and AEF are rare but potentially lethal complications that may arise following percutaneous or surgical radiofrequency ablation for (AF). The anatomic relationship between the left bronchus, the esophagus, and the posterior wall of the left atrium is changeable: the bronchus and esophagus are more susceptible to injury near the endocardial ablation areas. Bronchial and esophageal ulcers due to high temperature achieved by radiofrequency technology typically seem to precede fistula development. A high index of suspicion is recommended in patients who develop chest pain, fever, neurological deficit, and/or sudden hematemesis within days or weeks of AF ablation. Early detection by Computed Tomographic (CT) scanning is safe and feasible. Survival depends on rapid diagnosis and intervention; when untreated, the outcome is often fatal. In this study, two cases of ABF and AEF formation, respectively, arising after percutaneous and surgical radiofrequency ablation for chronic Rapid Atrial Fibrillation (RAF) are reported.

**Case 1:** A 35-year-old Caucasian man with a history of paroxysmal AF presented to the emergency room in Cefalù, Italy, due to repeated loss of consciousness, asthenia and pain, and tingling hyposthenia in the right upper limb 12 days after undergoing percutaneous radiofrequency ablation for AF. Brain CT and brain Magnetic Resonance Imaging (MRI) were normal. Laboratory studies were inconsistent with inflammation or sepsis; the patient demonstrated positive meningeal signs (i.e., neck stiffness and Brudzinski's sign.) The neurologist suspected viral encephalitis. Twelve hours later, the patient became comatose before subsequently dying due to serious heart rhythm disorders. Autopsy found a fistula between the left atrium, esophagus, and left bronchus with many septic emboli in the brain, heart, and kidney. The fistula walls showed coagulative necrosis with sites of acute suppurative inflammation and bacterial colonies.

**Case 2:** A 50-year-old Caucasian man with a history of paroxysmal AF presented to the emergency room in Cagliari, Italy, due to neurological deficit with tonic-clonic seizures and high fever arising one month after surgical radiofrequency ablation for AF. Laboratory studies revealed a slight leukocytosis, but the blood culture was negative. About two hours later, the patient died due to ventricular fibrillation. Autopsy found a fistula between the left atrium and esophagus and septic emboli in the brain and kidney. The fistula walls showed coagulative necrosis with sites of acute suppurative inflammation and bacterial colonies.

**Conclusions:** This study shows that adults presenting to emergency care with suspected meningitis, stroke, tonic-clonic seizures, disorders of consciousness and fever, and a recent history of radiofrequency ablation for AF (percutaneous or surgical) should be investigated. In these cases, the occurrence of AEF and/or ABF should be strongly considered. The diagnosis must be immediately confirmed by chest radiography or CT with contrast in order to promptly begin surgical therapy. In these two reported cases, the possibility of fistula formation wasn't considered despite the known history of radiofrequency ablation.

#### Radiofrequency Ablation, Fistula, Chronic Atrial Fibrillation



### H79 The Suitability of Cerebral Matter for the Forensic Identification of Highly Decomposed Bodies

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**Learning Overview:** After attending this presentation, attendees will have learned about methods and materials that are suitable and effective for the identification of highly decomposed bodies in forensic case work.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by clarifying how the identification of highly decomposed bodies in forensic case work is still a time- and resource-consuming topic. Therefore, further progress and research in this field is very important. The future goal is to develop a method for a fast and reliable method for DNA profiling of bodies where decomposition processes did reach advanced stages.

**Context:** When bodies are highly decomposed and identification based on morphologic features is no longer possible, Short Tandem Repeat (STR) genotyping has been proven to be a convenient and reliable alternative. But at very advanced decompositional stages, postmortem tissue putrefaction processes can have an effect on microsatellite instability; therefore, not every type of postmortem tissue is still suitable for STR genotyping and subsequent forensic identification. In many cases, bone or dental material is used, but such materials require elaborate reprocessing. Thus, multiple studies have focused on the eligibility of soft tissues and organs where DNA extraction is a lot easier and faster.

**Goals:** By evaluating different cases ( $n > 10$ ) in which successful forensic identification was only possible with DNA material extracted from specific tissues, effectively compare the differential suitability of soft tissues for DNA analysis in highly decomposed bodies.

**Material and Methods:** A systematic classification of decomposed bodies and analyzed organs was performed to evaluate the qualification of various soft tissues for DNA profiling in respect to the degree of decomposition. Swab samples were procured from the aorta, urinary bladder, brain, muscle tissue, liver, and oral cavity from bodies in a decomposition state with a Total Body Score (TBS) per Megyesi et al. ranging from 6 to 35 based on morphologic features.<sup>1</sup> Subsequent to DNA extraction, DNA concentrations and the DNA degradation status of the samples was evaluated. Finally, STR-marker profiles of each tissue sample were generated and compared.

**Conclusion:** Differences were detected in the suitability of various soft tissues for DNA-based identification. Cerebral matter turned out to be one of the most useful samples in highly decomposed bodies. Even in highly decomposed cases with a high TBS score, it is still possible to obtain a full STR-marker profile from brain tissue. Samples from the urinary bladder and the liver do not seem to be as stable for DNA profiling.

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#### Identification, Decomposition, DNA



### **H80 Acute Gastric Dilatation After Binge Eating: Overlapping Complications Determining a Race Against Death**

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**Learning Overview:** After attending this presentation, attendees will have meaningful insights on sudden death due to acute gastric dilatation, a condition frequently associated with eating disorders and often difficult to prevent.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through the report of fatal complications related to acute massive gastric dilatation subsequent to a binge-eating episode.

Acute gastric dilatation is a rare condition usually related to mechanical obstruction, neurological syndromes, or binge eating episodes. Although the stomach is known for being densely vascularized and resistant to ischemia, in rare cases the massive distension can cause a remarkable increase in intraluminal pressure and a significant vascular compression leading to circulatory insufficiency. Such a condition is usually followed by two main life-threatening complications: gastric wall necrosis and perforation.

This report presents the case of a 30-year-old woman with a clinical history of eating disorder who was transported to the emergency department with severe acute abdominal pain. Radiologic investigations documented subcutaneous emphysema, gastric dilatation, and a minimal layer of free air along the smaller gastric curvature. In light of these findings, emergent surgical intervention was requested. Despite the prompt diagnosis, the patient developed bradycardia and went into cardiac arrest refractory to resuscitation efforts just before reaching the operating room.

At autopsy, opening of the thoracoabdominal cavities revealed significant distension of the stomach, which occupied almost the entire left side of abdomen, displacing the small intestine. Prior to evisceration, 1,000cc of semi-liquid material that appeared consistent with gastric contents was recovered from the thoracoabdominal cavities. The stomach contained more than 3,000cc of partially digested material and was found to have a perforation along the smaller curvature. As a collateral finding, a prolapsed rectum extending about 15cm in length beyond the anus was found.

In this case, the huge food intake during a binge eating episode led to the development of acute massive dilatation of the stomach complicated by perforation. The peculiarity of the described case consists in the concomitance of multiple overlapping pathological conditions, which caused the death of the subject. Specifically, in addition to stomach perforation, abnormal gastric dilatation caused severe bradycardia—probably attributable to vagal stimulation—that resulted in irreversible cardiac arrest.

In conclusion, the rapid evolution of an infrequent disease and the superimposition of different pathological mechanisms precluded antemortem therapeutic interventions, thus requiring autopsy investigation to make a definitive diagnosis.

#### **Acute Gastric Dilatation, Gastric Perforation, Binge Eating**



### H81 Stent Thrombosis After Percutaneous Coronary Intervention (PCI) in Spontaneous Coronary Artery Dissection (SCAD)

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**Learning Overview:** After attending this presentation, attendees will better appreciate the issues related to no-reflow in revascularization treatment by percutaneous intervention in spontaneous coronary dissection.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reporting the major complications related to PCIs and their management in forensic pathology.

SCAD is a rare non-atherosclerotic cause of Acute Coronary Syndrome (ACS), accounting for 0.1% to 0.4% of all cases. SCAD occurs mostly in young women  $\leq 50$  years of age, and its causes are poorly understood and thought to be multifactorial with genetic, hormonal, and environmental influences. SCAD usually affect distal parts of coronary arteries and is caused by sudden disruption of the coronary arterial wall, resulting in separation of the intimal lining from the outer vessel wall and intramural hematoma formation. Pressure-driven expansion of the hematoma causes propagation of the dissection plane with formation of a false lumen containing thrombus. SCAD with active ischemia requires revascularization, preferably via stent implantation by PCI. PCI in SCAD has a success rate of  $< 50\%$ ; the major causes of morbidity and mortality after PCI are typically ischemic complications due to coronary dissection/hematoma propagation and stent thrombosis with abrupt vessel closure.

The presented report highlights the case of a 35-year-old woman admitted to the hospital emergency department with constrictive retrosternal pain radiating to the jugular area. Electrocardiogram (ECG) revealed pathologic waves in V1-V4, and laboratory tests showed an increase in myocardial enzyme levels (myoglobin 192.90U/L, total CK 1,053U/L, CK-MB 118U/L, LDH 639U/L). Coronary angiography revealed a spiral-shaped dissection in the Left Anterior Descending (LAD) coronary artery extending from the middle to the apex with a Thrombolysis-In-Myocardial-Infarction (TIMI) grade 2 flow. The following day, an ST-elevation (1mm) in V2-V5 was observed in the ECG (i.e., ST-Elevation Myocardial Infarction [STEMI]) and coronary angioplasty was performed with a subsequent decrease in myocardial enzyme levels and a remission of symptoms. After ten days, the patient again developed chest pain with an ST-elevation in the ECG (i.e., STEMI). Emergent PCI with implantation of a zotarolimus-eluting stent (3.5 x 16mm) was performed, but within 12 hours the condition of the patient rapidly declined and she expired.

An autopsy was performed to determine the cause of death. Gross examination of the heart revealed coronary vessels characterized by tortuous course and a bluish color. A palpable endovascular device was evident in the medial portion of the LAD coronary artery. Coronary arterial sectioning revealed an intimal dissection with a false lumen occupied by thrombotic material extending from 1cm after the origin of the LAD to the cardiac apex. Approximately 2cm after the origin of the LAD, a stent was found; the stent lumen, however, was completely occluded by thrombus. The thrombus originated from the stent and extended to the distal lumen of the LAD. Myocardial sectioning revealed an extended hemorrhagic infarction within the anterior wall of the Left Ventricle (LV) and the anterior interventricular septum.

In the presented case, stent thrombosis complicated PCI intervention; the presence of thrombotic endoluminal material in correspondence to the stent developed suddenly with resulting occlusion of the LAD coronary artery, resulting in an extensive myocardial infarction. The rapid evolution a relatively infrequent disease and possibility of different complications make any therapeutic intervention challenging—especially considering that current SCAD therapy is based upon opinion rather than randomized clinical trials. On the other hand, PCI results have also proven suboptimal in this challenging group of patients.

#### Coronary Artery Dissection, Percutaneous Coronary Intervention, Autopsy Investigation





## H82 The Relationship Between Wischnewski Spots and Stress Hormones During Hypothermia

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**Learning Overview:** After attending this presentation, attendees will know the main risk factors of hypothermia in Japan, and the hypothermia animal model could be expected to help clarify the mechanisms underlying Wischnewski spots in hypothermia.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying additional morphological and pathological findings in regard to hypothermia.

Numerous classical morphologic findings are commonly associated with the diagnosis of hypothermia, including Wischnewski spots (gastric erosions), color differences between left and right heart blood, frost erythema, and urinary retention. In general, risk factors for hypothermia include being a child or elderly, cold environmental conditions, wet clothing, being under the influence of alcohol or drugs, head trauma, decreased production of body heat, and malnutrition. There are many methods for creating a hypothermia animal model. For example, in the water bath model, a young animal is placed in a refrigerator at  $-20^{\circ}\text{C}$  until the core body temperature drops below  $35^{\circ}\text{C}$ . Then, the animal is placed in a Styrofoam™ box packed with ice. However, with such methods, morphologic features such as Wischnewski spots do not develop. The present study developed a hypothermia animal model based on risk factors and characteristic morphologic findings (including adrenal glands.)

Risk factor analysis was performed based on the records of autopsies performed between 2008 and 2018. Eighty-three deaths related to either hypothermia or secondary cold exposure were investigated. Sex, age, Body Mass Index (BMI), medical history, clothing, prescription medicine, urinary bladder and stomach contents, Wischnewski spots, blood alcohol level, detected drugs, and autopsy findings were reviewed. Out of 83 autopsies, 52 deaths were attributed to hypothermia and 31 involved secondary cold exposure. The decedents ranged in age from the high 60s to the 80s, with 26% more males than females. Regarding the BMI, 47% were underweight, and 43% were normal weight. Medical records showed that the most common condition in decedents was a history of high blood pressure ( $n=23$ ), followed by dementia ( $n=13$ ). Most cases showed evidence of paradoxical undressing ( $n=58$ ). Overall, no stomach contents were found in 76% of the cases, and Wischnewski spots were present in 92%. In addition, 45 cases in which Wischnewski spots were present showed evidence of being under the influence of alcohol. Postmortem toxicologic analysis showed evidence of opioids and barbitals ( $n=5$ ), benzodiazepines ( $n=4$ ), and tricyclic antidepressants ( $n=3$ ).

For the corresponding hypothermia animal model, Sprague Dawley rats were used and cold exposure conditions were created based on the human risk factors. Computed Tomography (CT) was used for postmortem imaging after cold exposure. The adrenal glands, stomach, and intestines were fixed in 4% paraformaldehyde embedded in paraffin. Each section was stained with the stress hormone corticosterone, hematoxylin, and eosin. With this animal model, Wischnewski spots were observed under the following conditions: an environmental temperature below  $5^{\circ}\text{C}$ , being under the influence of alcohol (2%), and if the animal model was hairless and the age group was 15~30 weeks. Fasting and water deprivation for three days resulted in the expression of Wischnewski spots involving more than 90% of the stomach; however, emphysema was not seen on Computed Tomography (CT). Regarding the immunohistologic findings, in the adrenal glands, corticosterone was mainly localized in the cytoplasm for the alcohol-induced, fasting, and dehydrated hypothermia model. Positive staining was seen in 60~80% of the zona fasciculata cells. Therefore, an animal model of hypothermia was established based on risk factors and morphological findings. Most of the classical morphological findings, including color differences between the left and right heart blood, retained urine contents, and the presence of Wischnewski spots, were visible in this animal model.

### Hypothermia, Animal Model, Risk Factors

### H83 New Biomarkers of Myocardial Necrosis Identification in Decomposed Bodies

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**Learning Overview:** In cases of prolonged postmortem interval, the forensic histopathological diagnosis is limited due to autolysis and putrefaction processes and detecting early Myocardial Infarctions (MIs) can be challenging. After attending this presentation, attendees will know two potential new biomarkers of myocardial necrosis identification in decomposed bodies: CD 68 and Myeloperoxidase (MPO).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that the autopsy has always had a critical importance as well as the histologic findings in MI diagnosis in medical malpractice cases. Using antibodies to human CD68 and MPO and their application in forensic fields may be a sensitive diagnostic tool to enhance accuracy of postmortem diagnosis of MI in putrefied bodies.

MI is one of the main sequelae of coronary heart disease and one of the most common causes of death worldwide.<sup>1</sup> Many medical malpractice claims are attributable to misdiagnosis, however.<sup>2</sup> Traditionally, the postmortem diagnosis of MI is made via histopathology using a combination of Hematoxylin-Eosin (H&E) staining and immunostaining on preserved heart tissue. In the case of decomposed myocardial tissue, immunohistochemistry plays a particularly important role in reaching the diagnosis of MI. In fact, although biological structures are predisposed to autolysis in the postmortem period, leukocytes and the nuclei of granulocytes are very resistant to autolysis and putrefaction.<sup>3</sup>

While there are many studies published in the scientific literature regarding postmortem MI diagnosis and timing of early ischemic injury, only a few studies have been performed on putrefied bodies. In these few studies, some immunohistochemical markers were found to improve diagnostic accuracy, including C5b-9 and NP57.<sup>4</sup> The current study examines two new biomarkers to determine whether they might potentially further improve diagnostic accuracy in cases of putrefied bodies.

Human CD68 is a transmembrane glycoprotein expressed in monocyte lineages and some other leucocytes. MPO is a heme-containing peroxidase stored in azurophilic granules of polymorphonuclear neutrophils and macrophages and released into extracellular fluid during inflammatory processes. These white blood cells are recruited immediately after an ischemic cardiac event. An immunohistochemical study was performed to evaluate morphologic changes in heart samples procured from eight autopsies performed on decomposed bodies after variable postmortem intervals ranging from 3 to 11 months. All were cases of presumptive medical malpractice due to the misdiagnosis of MI.

Control hearts from traumatic death cases did not show any immunoreactivity to MPO or CD68 markers. Immunohistochemical investigations of CD68 and MPO were able to confirm an MI diagnosis in all eight decomposed cases. Overall, these results suggest that autopsy plays a critical role in detecting histologic findings diagnostic of MI in potential medical malpractice cases. Using antibodies to human CD68 and MPO may increase postmortem diagnostic sensitivity in cases of suspected MI in putrefied bodies.

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#### Acute Coronary Syndrome, Ischemic Heart Disease, Myocardial Infarction



### H84 Viral Load: Handling an (Unexpectedly) High-Profile Case

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**Learning Overview:** After attending this presentation, attendees will be aware of what can happen when a case goes unexpectedly viral and how to approach an unexpectedly high-profile case.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of coroners, medical examiners, and forensic investigators of how a seemingly routine case can capture the public's attention, how that interest can affect the office and personnel involved, and how medical examiners' and coroners' offices can be prepared to respond to the increased public scrutiny.

Sometimes it is easy to anticipate when a case is going to be high profile. Police-involved shootings, deaths in custody, deaths of celebrities, and deaths in public places frequently get discussed and dissected in the news and on social media. In such instances, there is often an increased awareness among the death investigation team at the time of notification of death and at autopsy to make certain that any reasonably foreseeable questions regarding the circumstances, and cause and manner of death, are addressed. Measures can be taken beginning at the time of autopsy to anticipate and respond to the expected public scrutiny so that everyone is prepared for the intense interest. But what happens when a case unexpectedly captures the fascination of the public?

Such a case was investigated by the Cook County Medical Examiner's Office. A scene investigation was performed according to office protocol and the autopsy was completed within hours of discovery of the deceased. By that evening, there were already posts on social media and in the news about the death. As the death investigation progressed, so did public involvement as various individuals evaluated the information provided by the media and by online information, videos, and photographs posted by friends and acquaintances of the deceased.

There were protests, social media rants, and YouTube® videos analyzing the investigation and those involved. Attempts at transparency were met largely with suspicion, more demands for answers, increased lay interpretation of evidence, and efforts to become involved in the investigation.

After a discussion with the family, the Medical Examiner's Office expedited closure of the case and issued a formal press release. But closing the case neither stopped the demands for answers nor diminished allegations of cover-ups, which accused police and medical examiner personal of complicity in foul play. Demands for justice came from around the world through letters, emails, telephone calls, Facebook® messages, Tweets, and visits to the office.

Who knows why a case goes viral? In this age of the "CSI effect," social media, armchair detectives, and lay sleuths, this case provides an example of how forensic pathologists and offices can best deal with the unexpected media and popular scrutiny, and what their responsibility is to respond to those requests.

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### High Profile, Social Media, Autopsy



### H85 Increasing Transparency: The Utility of Layered Images in Postmortem Identification

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**Learning Overview:** After attending this presentation, attendees will be able to utilize a simple method of comparing radiographic images to identify remains. The goal of this presentation is also to present rationale for medical examiner access to electronic health records.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the usefulness and reproducibility of radiographic comparison for identification.

Accurate identification of decedents is of paramount importance in medicolegal death investigation. There are multiple well-recognized methods of identification with the most utilized being direct visual identification by family members or close friends, which is often quick and accurate. In some circumstances (fire-related deaths, decomposition, no known next of kin, etc.), visual identification cannot be accomplished. Fingerprint identification is generally as rapid as the availability of the agency that performs the comparison, but lack of antemortem or postmortem prints may prevent such identification. DNA comparison introduces significant delays, and dental records can be challenging to acquire.

Electronic access to antemortem radiographs may prove useful, timely, and accurate as a comparison tool for postmortem radiographs. In cases of unidentified remains at the Jefferson County Coroner/Medical Examiner's Office, seven decedents were identified using a novel and simple technique of superimposing images captured from both the postmortem radiograph and antemortem records. The superimposition (or layering) was performed using an easy method in Microsoft® Word. The transparency of the overlaid image was adjusted to ensure skeletal features were consistent (if not identical) in both radiographs. Often the medical examiner had access to medical records on the day that the decedent was examined, which drastically improved the time to identification (usually one to three days) and release of remains to family members.

Traditional methods of acquiring physical Computer Discs (CDs) containing digital images usually involves facsimile requests to local hospitals, delivery by mail or in person, and review of image files with appropriate software. This process resulted in significant delays for establishing identification (5 to 41 days in this cohort). In either scenario (electronic access vs digital image from CD), many skeletal landmarks, including frontal sinus morphology, vertebral shapes, and pelvic and femoral shape, were used.<sup>1-4</sup> Of course, this radiologic comparison was used in conjunction with other circumstances from the case to conclusively identify the decedent. The resultant Microsoft® Word document can become a physical visual aid that is a permanent record within the case file and could provide a useful tool in courtrooms and meetings with individuals not accustomed to comparing side-by-side radiographic images.<sup>5</sup> The practice of capturing images for comparison is limited by the paucity of plain radiographs available in the modern medical record. Computed Tomography (CT) scans are the mainstay of clinical imaging and are difficult to compare to postmortem radiographs, unless evidence of surgery or bony trauma is obvious. However, offices with CT scanning capability may be able to use this same layering technique with antemortem CT images to aid in identification of remains.

This case series presents a novel strategy for timely and accurate identification, as well as highlighting the importance of a medical examiner's office having access to the electronic health records of local hospitals.

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#### Identification, Radiology, Forensic Pathology



### H86 An Interdisciplinary Approach to Data Collection of Unidentified Juvenile Remains at the Georgia Bureau of Investigation (GBI)

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**Learning Overview:** After attending this presentation, attendees will understand some of the challenges facing the identification of juvenile skeletal remains and learn about the effectiveness of a multi-step protocol for the collection of biometric and biological data from juvenile remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showcasing how an interdisciplinary approach in the medical examiner's setting is vital to aiding in the identification of a large collection of unidentified individuals.

The GBI houses the skeletal remains of unidentified individuals ( $N=272$ ) dating back to the 1960s. Legislation was passed in 2016 to allow for the burial of these individuals. Prior to internment, it was decided that a thorough examination of every case was necessary to create a robust case file in the event that a lead on identification should arise. Of these cold cases, anthropological analyses were used to determine that approximately ten percent ( $n=26$ ) were juvenile (e.g., skeletally immature). Long bone length, the appearance and union of epiphyses, and the development and eruption of the dentition were used to approximate the age at death of each individual, ranging from 20 weeks *in utero* to 25 years of age.

The identification of juvenile remains is particularly difficult because many of the other standard components of an anthropological analysis, such as biological sex and ancestry estimation, are less reliable before skeletal maturity. Further, the condition of these remains varies. While some cases are largely complete skeletons, others are comprised only of bone fragments or else the present bone is in extremely poor condition due to weathering or burial. As a result, a uniform approach to analyses would be ineffective. In response, this study developed a diverse, multi-step, interdisciplinary protocol to collect a large range of biometric data and biological specimens from each individual before internment. Each set of remains was photographed, inventoried, and analyzed using gross morphological techniques. Metric measurements of all present elements were also collected. In addition to traditional DNA comparison, bone samples were extracted for stable isotope analysis. Unique isotopic signatures (carbon, nitrogen, oxygen, and strontium) in bone may be distinguished based on regional differences in drinking water supply. For individuals in which the cranium was fully formed, crania were scanned at 0.5mm intervals with a peripheral Quantitative Computed Tomography (pQCT) scanner at Kennesaw State University. The completed Digital Imaging and Communications in Medicine (DICOM) stacks will subsequently be used to print 3D models for forensic artistic reconstruction. While all cases in the GBI's unidentified collection will receive a thorough examination, this study argues that this multi-step data collection protocol using new technological developments is key to the potential identification of juvenile individuals.

#### Unidentified, Juveniles, Anthropology



### H87 Lost and Found: Forensic Anthropology and the Recovery of a 21-Year-Old Plane Crash

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**Learning Overview:** After attending this presentation, attendees will understand how forensic anthropologists can assist medical examiners and forensic pathologists in complex scene recoveries involving skeletal remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the expertise forensic anthropologists provide in recoveries of highly fragmented skeletal remains that may be found in medicolegal death investigations.

This presentation will review the case of a 21-year-old plane crash in which the Medical Examiner of Mackinac County, MI, consulted with forensic anthropologists for the recovery of highly fragmented human skeletal remains. The remains were presumed to be Mark and Janet Davies, a married couple who disappeared on September 14, 1997, when their personal plane left Drummond Island, MI, with the intention of traveling to the Livingston County Airport, MI. The forensic anthropology teams from Northern Michigan University and Western Michigan University Homer Stryker M.D. School of Medicine assembled in St. Ignace, MI, on August 7, 2018, at 8:00 a.m. to partake in a planning meeting with teams representing the United States Forest Service, Mackinac County Medical Examiner's Office, Mackinac County Sheriff's Office, Federal Aviation Administration, National Transportation Safety Board, and Piper Aviation. The scene, discovered by United States Forest Service interns conducting a tree survey, was located within the Hiawatha National Forest, approximately half a mile from the nearest trail.

The plane was dismantled in sections from the top working down and the various teams of investigators cooperated to ensure that everyone had the opportunity to meticulously collect evidence relevant to their respective disciplines. Skeletal elements were recovered from the control panel and footwell of the cockpit, as well as from the soil just outside the fuselage. The anthropology team, accompanied by deputies from the Mackinac County Sheriff's Office, returned on August 8 to continue the recovery and excavated additional skeletal material from the surrounding soil. The remains were transported to Western Michigan University Homer Stryker M.D. School of Medicine's Forensic Anthropology Laboratory, where skeletal analyses were performed. Evidence of blunt force trauma consistent with a plane crash was present, including a compression fracture of the first cervical vertebra, spiral fractures, and butterfly fractures of various skeletal elements. A minimum number of two individuals was established based on repeated elements. Commingling was resolved by separating repeated elements and comparing sexually dimorphic size differences in the elements recovered. Taphonomic analysis revealed animal scavenging activities and weathering. Comparative dental radiography confirmed the decedents to be Mark and Janet Davies. After the completion of the skeletal analyses, the remains were returned to Mark's and Janet's next-of-kin. Remains that could not be reliably associated with either Mark or Janet Davies due to extreme fragmentation were slated for cremation together, an arrangement that was agreed upon by both families.

This unique case highlights the need for interdisciplinary teams within the forensic sciences. As forensic anthropologists are experts in the recovery and analysis of human skeletal remains, they can be valuable in difficult cases where fragmentary skeletal remains need to be located and recovered. In this case, the forensic anthropologists provided expertise in the search and recovery of highly fragmented skeletal remains, the analysis of skeletal trauma, the resolution of commingling, and the positive identification of the victims.

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#### Forensic Anthropology, Scene Recovery, Forensic Science



### **H88 The Minnesota Protocol: A Standardized Postmortem Investigation Guideline**

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**WITHDRAWN**



### H89 The Complicated Recovery of a House Fire Victim in Northern Michigan

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**Learning Overview:** After attending this presentation, attendees will understand how forensic anthropologists can assist medical examiners and forensic pathologists in scene recoveries involving fragmentary, thermally altered skeletal remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the value of involving forensic anthropologists when recovering highly fragmentary skeletal remains as part of a medicolegal death investigation.

This presentation will review the recent recovery of fragmentary human skeletal remains from the scene of an intense, unwitnessed house fire in northern Michigan. Michigan's Upper Peninsula (UP) is known for its extensive Great Lakes shoreline, extreme winter weather, and a low population density that allows residents and visitors to disconnect from the bustle of life in larger cities and enjoy relative isolation. The overall population density changes seasonally, with far fewer people residing in the UP in winter than in summer. In February of 2019, a single-family residence caught fire. Because the nearest residences were unoccupied at the time and the nearest neighbor was over one-half mile away, the fire went unwitnessed and unreported until it had essentially burned itself out. The residence was known to have been a single-story ranch-style house with a full basement, owned and occupied by a single homeowner. Extremely fragmentary, calcined human skeletal remains were located in an area of the lower level, indicating the victim had been on the main floor but had fallen into the basement when the floor collapsed during the fire. No foul play was suspected by investigators.

Extreme winter weather conditions and unsafe temperatures following the fire complicated the investigation. Overseen by the Mackinac County Medical Examiner, the recovery of human skeletal remains from the basement was primarily conducted by law enforcement and fire personnel. The investigators did their best at the time, but the onset of heavy snow during this initial recovery obscured visibility of both the remains and any potentially hazardous conditions in the home. By the next day, approximately two feet of snow covered the scene (and snow continued to accumulate for the next several weeks). Further recovery was halted, and the medical examiner transferred custody of the remains to the Forensic Anthropology Research Laboratory at Northern Michigan University (NMU) for analysis. Anthropologists were asked to determine the minimum number of individuals, analyze the skeletal trauma, provide a biological profile, and identify the victim. Identification at that time was complicated due to the fragmentary and incomplete nature of the remains, as only approximately 50% of the body was represented, resulting in the absence of comparable features for comparative radiography.

Approximately three months later, when the snow finally melted and the scene was once again visible, the medical examiner and local law enforcement initiated a second excavation to complete the recovery of the victim. The medical examiner, recognizing the need to work with skeletal specialists on a case involving such fragmentary, extensively burned remains, called upon the NMU anthropologists to assist with this phase of recovery. The NMU anthropologists methodically excavated the area of the basement where the victim was known to have been located, resulting in the location of skeletal elements representing all regions of the body that were missing following the first recovery, many of which had not been recognized as bone during the initial recovery effort due to extreme thermal alteration and difficult visibility. As a result of the additional excavation, a positive identification was made using comparative dental radiography and the medical examiner was able to issue a death certificate.

This case highlights the complicated nature of forensic recovery of human remains from scenes that involve adverse conditions, such as fire damage and extreme winter weather, as well as the need for collaborative, interdisciplinary teams. The forensic anthropologists in this case provided much-needed expertise in skeletal identification and excavation. The medical examiner considered this service vital for both victim identification and to be certain no remains were left behind in the ruins.

#### **Burned Remains, Scene Recovery, Forensic Anthropology**





## H90 Selfie-Related Injuries and Deaths in Italy

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**Learning Overview:** After attending this presentation, attendees will be aware that the incidence of taking extreme selfies to be shared on social media is increasing, also leading to selfie-related trauma and death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting that the incidence of selfie-related injuries and deaths is not negligible worldwide and is a problem that also cannot be ignored in Italy.

According to the Oxford dictionary, a selfie is defined as “A photograph that one has taken of oneself, typically one taken with a smartphone or webcam and shared via social media”. Clicking selfies and sharing them on social media has become a common means of self-expression. However, individuals sometimes portray themselves in dangerous settings so as to gain attention on social media sites. In some cases, this may have fatal consequences. A “Killfie” is actually a term used in the common language to define an extreme selfie which is potentially life-threatening for the photographer. Cases of selfie-related death or mortality are defined as accidental deaths precipitated by the process of self-photography; these may occur just before, during, or just after the process, which is typically documented via cell phone. This worldwide phenomenon also occurs in Italy, though incidence has not been well established, and scientific medical journal articles on the subject are lacking.

In order to better define the exact incidence of selfie-related injuries and deaths in Italy, a media-based search from 2014 to 2019 was performed; medium age, geographical locations, and manner of occurrence were analyzed and compared with other media-based searches in the literature from other countries.<sup>1,2</sup>

Study results suggest that information and awareness campaigns are necessary, especially among the youngest: in India, for instance, “no selfie zones” have been created, while in Russia, a campaign urging people to take safer selfies has been launched. It is probable that there is an underestimation of the real number of selfie-related injuries and deaths in the studies conducted worldwide; in fact, other studies, by considering only English language media articles, do not take into consideration the local press written in other languages. Researchers worldwide should therefore conduct similar studies in order to describe this phenomenon in a thorough manner in all nations, to obtain an actual count of selfie-related injuries and deaths.

With regard to selfie-related deaths, the forensic pathologist’s role entails shedding light on the dynamics of the events; although the cause of death is rarely in doubt, the manner of death is often unclear. Consequently, the inquiry needs to be thorough to establish if the death was indeed accidental rather than suicidal or homicidal in nature.

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### Selfie, Selfie-Related Death, Selfie-Related Injury



### H91 The Introduction of a Mobile Application to Assist in the Evaluation and Investigation of Death Scenes in Forensic Medical Practice

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**Learning Overview:** The goal of this presentation is to discuss the development of a mobile app designed to function on smart phones and tablets, to assist Medical Examiners (MEs) and death investigators in the systematic evaluation and contemporaneous recording of appropriate findings at a death scene. Other ME offices and those officials involved in medicolegal investigation of death may benefit from this overview.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing/proposing the use of a relatively simple but functional mobile app to ensure contemporaneous and standardized collection and recording of information at the death scene, which can then be relatively easily shared with other parties at distant locations.

The introduction of mobile application technology may substantially assist in ensuring that relevant and standardized information is systematically and contemporaneously captured, stored, and made available for assessment by trained professionals, thereby assisting in eventual criminal and civil justice administration. This tool may have potential for significantly improving the quality of medicolegal death investigation, not only in South Africa, but internationally.

Thorough medicolegal death/crime scene investigation—and the recording of relevant findings—plays a critical role in the overall understanding and elucidation of non-natural deaths. Information gathered at the scene of death assists in planning the subsequent death investigation (including, in particular, the autopsy and related special investigations, such as toxicology) and thus ultimately may play an important role in establishing and validating the cause and manner of death.

By applying commonly available smart phone/tablet technology in the form of an application (“app”) to the death scene, a simple yet effective aid to death investigation has been developed at the Department of Forensic Medicine of the University of Pretoria for routine use by death investigators and pathologists. This application allows for structured, rapid, and real-time capture and storage of relevant information in electronic format (including photographs), which can in turn be immediately made available by means of wireless transmission technology to off-site parties, including consultant pathologists, police officers, etc. Security of information is ensured by appropriate data encryption technology.

A multiplicity of relevant facts or information fields may be easily and accurately incorporated into the on-site data capture, including elements such as crime/death scene Global Positioning System (GPS) coordinates, ambient temperature, scene photographs, etc. The information can then be transmitted and stored in electronic format and/or linked to existing (or subsequent) data archives. This application is specifically geared toward supplementing or enhancing the information fields/sets that forensic pathologists may require or find to be of value in order to prioritize scene attendance, plan and conduct their postmortem examinations or viewing procedures, or to interpret subsequent autopsy findings. The tool may particularly benefit countries or communities where there is a lack of adequately skilled or trained death scene investigators—which may indeed compromise the quality of the initial investigation (and even lead to grave miscarriages of justice).

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#### Mobile Application, Death Scene Investigation, Forensic Pathology



### H92 An Evaluation of Selected Hematopoietic Immunohistochemical Stains in Decedents in Varying States of Decomposition

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**Learning Overview:** The goal of this presentation is to present data on the utility of postmortem Immunohistochemistry (IHC) in decedents in varying states of decomposition. IHC is often utilized in the surgical and cytopathology realms but is less commonly used in autopsy pathology. This study sought to discover if the quality of immunohistochemical stains decreases with increased decomposition. This will allow institutions to make decisions regarding the benefit of performing the selected stains evaluated in this project on decedents with prolonged death intervals and/or significantly autolyzed tissues.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing utility data to practitioners considering performing postmortem hematopoietic immunohistochemical stains.

**Hypothesis:** The quality of immunohistochemical staining decreases with increased autolysis.

**Methods:** Mediastinal lymph nodes were collected prospectively from ten autopsy cases at The University of Texas Medical Branch. The postmortem interval ranged from one to five days. The lymph nodes were fixed in 10% formalin and submitted for routine histologic processing. Each lymph node was stained with Hematoxylin-Eosin (H&E), CD45, CD5, CD20, CD79a, and CD2. Lymph nodes and the IHC markers were chosen for several reasons: (1) approximately 12 cases of lymphoma are diagnosed at UTMB postmortem (no pre-mortem diagnosis available) making lymph nodes a very important postmortem source for hematopoietic markers; (2) mediastinal lymph nodes are more centrally located than peripheral tissue and are thus less susceptible to rapid heating and cooling from transport and storage of bodies; (3) mediastinal lymph nodes are easy to identify at autopsy; and (4) membranous stains were chosen to minimize the number of variables encountered by the reading pathologists.

The signal intensity and quality of the IHC stains were scored by three board-certified pathologists (i.e., observers) including: two autopsy pathologists and one hematopathologist. Average scores for each stain in each case were calculated. These scores were plotted to compare degree of autolysis (represented by H&E average scoring) against quality of IHC staining (represented by the average score of each IHC stain in each case). Inter-Observer Variability (IOV) was assessed using the weighted kappa statistic.

**Results:** The postmortem interval did not correlate with the degree of autolysis observed in the histologic sections, but observers rated the degree of autolysis seen in each H&E stained section (scale: 1=normal histology with no sinus histiocytosis; 5=severe autolysis) before reading each associated immunohistochemical IHC stain. For the degree of autolysis seen in each H&E section, IOV kappa score was moderate to substantial ( $k=0.444-0.653$ ). A line plot of average scores for both H&E and IHC showed that as the degree of autolysis increased, the quality (signal intensity and completeness) decreased. A line plot of average scores for H&E compared to average weighted kappa values for each case showed no appreciable change in IOV regardless of H&E scores. That is, the degree of autolysis did not appear to affect the observer agreement scores ( $m=-0.1159$ ). IOV kappa score for IHC was highest for CD79a ( $k=0.6$ ), followed closely by CD20 ( $k=0.595$ ). The lowest IOV kappa score was for CD45 ( $k=0.312$ ).

**Conclusion:** Though IHC is not commonly used in forensic pathology, it is generally available to the forensic pathologist who needs it. IHC is more frequently used in the hospital autopsy setting than in the forensic setting, but its utility for tissues with varying degrees of autolysis has been only rarely studied. This study confirms the prevailing belief among pathologists that usefulness of IHC in the autopsy setting decreases with increased tissue autolysis.

#### Autopsy, Immunohistochemistry, Lymphoma



## H93 “Forens-OMICS”: The Application of Omics Sciences to Forensic Investigations

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**Learning Overview:** After attending this presentation, attendees will understand the potential of “omics” methodologies to estimate Postmortem Interval (PMI). Attendees will be introduced to “Forens-OMICS” as the first global application of several “omics” technologies to forensics and will discuss current state and preliminary findings from omics data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing an innovative approach to PMI estimation. Combining molecular methods with visual observations may provide more precise and unbiased estimates and help overcome current methodological limitations.

Estimates of time-dependent biological processes (e.g., PMI and age at death) are based primarily on visual observations and require considerable experience and expertise. Moreover, most methods rely on the completeness of remains or presence of certain skeletal elements. This limits the possibility to obtain precise estimates from incomplete remains, a common situation in mass disasters, conflicts, terrorist attacks, and routine casework.

Biomolecules, such as proteins and lipids, are abundant in bone tissue and persist long after death. Consequently, these biomolecules may retain and reveal information about time-dependent biological processes significant to forensic investigations. Proteomics provides information about age at death and PMI, and lipidomic analyses of skeletal muscle and bone marrow are useful for PMI estimation.<sup>1-5</sup> This presentation demonstrates an integrative approach using various sources of omics data to reveal quantitative biomarkers for estimating PMI and age at death from remains in advanced states of decomposition, including skeletonization.

Omics data were obtained from four sources: pig bones, mice bones, human bones, and human skeletal muscle tissue. Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) was used to identify biomarkers on pig ( $n=4$ ) and mice carcasses ( $n=96$ ) of prolonged PMIs (up to six months). Proteomic studies identified biomarkers suitable for PMI, as well as age-at-death estimation (e.g., fetuin and biglycan). Lipidomics analyses of human muscle tissue from 16 human bodies at the University of Tennessee Anthropological Research Facility ( $n=262$  samples, Accumulated Degree Days [ADD] 0–2,000) were analyzed via MS/MS. Cell membrane glycerophospholipids, specifically phosphatidylglycerol and phosphatidylethanolamine, showed the highest predictive power of PMI (R-square=0.85). Lipidomic analyses of human trabecular bone from three skeletal sites (calcaneus, proximal tibia, vertebral body) were conducted on 135 individuals. The sample consists of a cross-sectional and longitudinal component: (1) 115 skeletons from the William M. Bass Donated Skeletal Collection (PMI=1–30 years); and (2) 20 individuals placed at the University of Tennessee Anthropological Research Facility in 2018, which are being sampled every six months for two years. Analyses with a high-resolution electrospray ionization lipidomics analytical platform have identified 76 potential N-acyl amino acids; two have been validated via generation of the MS2 product ion for serine (palmitoyl and oleoyl serine).

These preliminary results show that omics data have great potential for transforming current approaches for estimating time-dependent biological processes of forensic import (e.g., PMI and age at death). Further lipid and protein class candidates are being validated with MS/MS, and machine learning algorithms will be used to analyze the combined omics data in addition to other omics results (e.g., DNA methylation data from bones) with the goal of improving predictive power and identifying the most accurate methodology to assess time-dependent forensic parameters.

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### Omics Technologies, Postmortem Interval, Age Estimation



### H94 Proteogenomics: Shifting Touch Sample Analysis Paradigms

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**Learning Overview:** After attending this presentation, attendees will understand that it is possible to isolate both DNA and protein from human touch samples, providing a second meaningful source of genetic information on which forensic analysis can be based, in addition to traditional DNA analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by aligning with the competencies of attendees who process trace forensic samples. Proteomic analysis is rapidly maturing, providing value to attendees focused on performance who are looking to implement these methods to derive additional value from these challenging sample types. These results demonstrate that this approach permits traditional DNA analysis and parallel protein analysis, highlighting the relevance to both the core competencies and performance requirements of typical attendees.

Human touch samples represent an increasing fraction of forensic casework analysis each year. Unfortunately, these samples are also among the most difficult to analyze effectively, especially with respect to traditional DNA analysis. Trace DNA in touch samples commonly leads to marker drop-out and other artifacts and can complicate analysis of DNA mixtures. Certain evidence types, such as brass shell casings, may further contribute to DNA degradation or inhibition. Proteins represent a separate, often overlooked, class of biomolecules in touch deposits that are both highly abundant and environmentally robust. The use of mass spectrometric-based proteomic analysis to identify Genetically Variable Peptides (GVPs) has been recently demonstrated to provide forensically relevant match probabilities to suspected individuals.

This study describes the application of a novel complete casework-oriented proteogenomic analysis method that encompasses sample preparation, DNA profiling, proteomic analysis, and a GVP-specific Random Match Probability (RMP) calculator specifically designed to use proteogenomic data sets. Using both human fingerprint samples as well as artificial fingerprints to standardize DNA and protein inputs, the study demonstrates that this approach maximizes DNA and protein yield across multiple surface types, including glass, plastic, laminate, and brass. Critically, the sample collection and extraction procedure stratifies and differentially extracts DNA and protein fractions, enabling parallel DNA and GVP analysis without significant loss of either fraction. This capability promotes future casework laboratory implementation by positioning GVP analysis as a complimentary tool and enhancement to standard DNA analysis (as opposed to promoting GVP analysis as a replacement of the current gold standard). Using this novel method, the current study demonstrates improved DNA yield in comparison to standard extraction methods. By extracting and analyzing protein markers from the same sample, these results further show that humans can be identified from touch samples with RMP values ranging from  $10^{-5}$  to  $10^{-16}$  regardless of DNA content. Taken together, this novel sample preparation strategy and custom analysis method provides a transformative capability for the analysis of touch samples.

#### Forensic Genomics, Forensic Proteomics, Touch Sample Analysis

## H95 Developing Biological Models for the Probabilistic Genotyping of Next Generation Sequencing (NGS) Data

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**Learning Overview:** The goal of this presentation is to explain the need for models that describe the behaviors of NGS DNA profiles and the methods for developing said models for probabilistic genotyping software.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing current efforts in the modeling of NGS profiles.

Conventional Polymerase Chain Reaction (PCR) -based amplification of Short Tandem Repeats (STRs) at different discriminatory loci, and subsequent fragment characterization using Capillary Electrophoresis (CE), is currently the predominant DNA profiling approach used in forensic laboratories. Early methods for the interpretation of the resulting electropherogram (epg) profiles were very limited, utilizing a binary approach. This binary interpretation was largely threshold-based and did not account for the probability of drop-in and drop-out of alleles in a DNA profile.<sup>1</sup>

As technology developed, DNA profiles increased in complexity with more sensitive DNA profiling methods, resulting in more mixed DNA profiles. This resulted in the need for probabilistic genotyping methods. This is where additional information, such as peak heights and stutter peaks, present in an epg is also considered in the interpretation of a profile.

The analysis of forensic DNA methods using STR and CE methods have been used within forensic DNA laboratories internationally for more than 20 years.<sup>1</sup> More recently, laboratories have started investigating NGS methods for analysis of forensic samples. There are known limitations with a CE-based approach that NGS methods may be able to resolve.<sup>2</sup>

An example of the benefits of an NGS approach is its capability of resolving iso-alleles. These are alleles with the same number of STRs, but with different DNA sequences.<sup>3</sup> Using the sequence information within these iso-alleles, different repeat patterns of the same repeat motif(s) can be observed, allowing for increased discrimination of profiles and possibly improved resolution of contributors.<sup>4</sup>

Analogous to how interpretation methods were limited during the early implementation of CE-based technology, the current methods for the interpretation of DNA profiles obtained through an NGS approach are limited. There have been examples in which existing probabilistic genotyping software, which is developed for the interpretation of CE DNA profiles, is modified to interpret NGS DNA profiles.<sup>5</sup> The assumption is that the models that exist within these probabilistic genotyping software are suited for the interpretation of NGS. There is an increasing need for the development of more sophisticated biological models for use within probabilistic genotyping software for NGS DNA profiles.

In order to develop biological models for the interpretation of NGS DNA profiles, it is first necessary to understand how these profiles behave. This presentation explores the behavior of NGS DNA profiles, including aspects such as stutter, peak height variability, and locus-specific PCR efficiency. The goal is to identify the similarities and differences between CE and NGS DNA profiles. Experiments designed to determine these models and findings are presented.

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### NGS, Probabilistic Genotyping, DNA



### H96 Decomposition Odor Production in a Tropical Savannah

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**Learning Overview:** After attending this presentation, attendees will understand the relation of the Volatile Organic Compound (VOC) profile emitted from decomposing remains in a tropical climate to those in other climate regions.

**Impact on the Forensic Science Community:** This presentation will benefit the forensic science community by providing information about the reproducibility of decomposition odor for a new and common climate region. This is crucial to the courtroom defense of applications that rely on the detection of decomposition odor for search and recovery.

Cadaver detection canines perform an important role in the search and recovery of human remains in missing persons cases, homicides, and mass disasters. In recent years, significant advances have been made in the analytical tools used to profile the VOCs constituting the target odors for cadaver detection canines. It is well known that these canines are highly efficacious, especially in scenarios that involve a large search area or challenging terrain. They are also capable of locating human remains in a variety of weather conditions and climates across the world. However, decomposition odor research has only been performed in select locations in the past, and, therefore, more detailed information is required to understand what is consistent to odor evolution regardless of the local environment. The purpose of this study was to perform the first decomposition odor study in a Tropical region, specifically the Tropical savannah climate experienced in Honolulu, HI. Honolulu is a contrasting location to areas that have been previously studied due to relatively consistent and warm temperatures year-round, as well as minimal temperature differences throughout the day. Tropical savannah is also the world's second most common climate type (following Hot desert), and these regions experience many climate events, such as tropical storms, that may contribute to a need for forensic search and recovery. Therefore, it is very important to improve our understanding of decomposition odor in these regions. The hypothesis was that a major portion of the VOC profile would be comparable to previous studies performed in other environments.

Three pig (*Sus scrofa domestica*) carcasses were placed on an exposed soil surface and decomposed for two weeks. Every second day, the pig carcasses were sampled for VOCs by covering them with a stainless steel hood and pumping air from above the carcasses onto a sorbent tube. Additional VOC samples were also collected from larval masses at each carcass during the period that they appeared. Sorbent tubes were thermally desorbed and analyzed using comprehensive Two-Dimensional Gas Chromatography/Quadrupole Mass Spectrometry/Flame Ionization Detection (GC×GC/qMS/FID). Approximately 30 compounds were tentatively identified from the decomposing remains, the majority of which have been reported in previous literature. This demonstrates consistency in the decomposition VOC profile in a new geographical region. Temporal trends were tracked for several compounds prominent in literature, such as alcohols, aldehydes, ester, ketones, and sulfur-containing compounds, and longitudinal trends were confirmed to present similarly to past studies. In addition, larval mass samples were found to contain rich VOC profiles, with high abundance of compounds known to be present in the decomposition VOC profile (e.g., 2-butanone, dimethyl disulfide, and 1-butanol). Larval mass data may assist in understanding the metabolic contribution of larval masses to the overall decomposition odor profile.

This research is significant because it is the first study to establish the decomposition odor profile in a Tropical savannah. This supports the development of a core VOC profile that will help provide a chemical foundation for the highly efficacious use of scent detection canines. Such information is essential to the courtroom defensibility of biological detectors, such as canines, as well as the development of chemical sensors that may help in the future to assist in large-scale search and recovery efforts where resources may be limited.

#### Forensic Taphonomy, Scent Detection Canines, Volatile Organic Compounds



### H97 Comparing Resolution of Mixtures by DNA Sequencing Using the Illumina® MiSeq® FGx System

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**Learning Overview:** The goal of this presentation is to show that the MiSeq® FGx system is concordant with current Capillary Electrophoresis (CE)-based analysis and provides additional sequencing data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing a possible alternative to CE-based Short Tandem Repeat (STR) analysis that can provide additional sequencing information to mixture samples.

The use of STRs for genotyping forensic case samples has long been an effective tool for human identification. However, deconvolution of forensic STR mixture samples can be difficult and being able to obtain additional information to aid in this process will be important. Allele overlap and stutter during PCR can cause drop-out of the minor contributor's alleles and result in incorrect allele calling. The GlobalFiler® Polymerase Chain Reaction (PCR) Amplification kit targets 21 autosomal STRs, amelogenin, and 2 Y-indels. In comparison, the ForenSeq™ Signature Prep Kit on the MiSeq® FGx targets an additional 6 autosomal STRs, 24 Y-chromosomal Short Tandem Repeats (Y-STRs), 7 X-chromosomal Short Tandem Repeats (X-STRs), and 94 Single Nucleotide Polymorphisms (SNPs), and it also provides the DNA sequence of those targets. The additional loci as well as separation of alleles by sequence should provide much more information for resolving mixture samples. The contributor ratio accuracy and MiSeq® FGx performance is analyzed here and compared to current CE-based methods. The DNA sequencing process used here requires three PCR amplification steps overall. Additionally, there are many wash steps and transfer steps involved in the purification and normalization of the libraries prior to sequencing. Together, the number of steps may increase profile variability.

A side-by-side assessment of the ForenSeq™ Signature Prep Kit with the MiSeq® FGx system and the GlobalFiler® PCR Amplification Kit using equivalent samples containing two-person DNA mixtures at three different mixture ratios is presented. The ratio of two contributors was calculated at three mixture ratios (1:1, 1:4, and 1:9) to use as a means of comparison. Each mixture was analyzed in quadruplicate twice on two separate amplifications. The mean contributor ratios calculated on the MiSeq® FGx were 1.799, 7.595, and 13.524 for the 1:1, 1:4, and 1:9 mixtures, respectively. This was not significantly different from the CE mean contributor ratios of 1.818, 7.722, and 14.827, respectively. There was a total of 68 minor contributor alleles (9.09% of the total possible alleles) that were lost on the MiSeq® FGx™ and only 20 on the CE (3.38% of the total possible alleles). Most of those alleles lost on the MiSeq® FGx (61 or 8.15%) and CE (18 or 3.03%) were from the 1:9 mixtures. This difference may seem large, but there were an additional five minor contributor alleles, per replicate, that could be identified by sequence. A high preferential amplification of the D22S1045 was seen, which resulted in the loss of that locus for contributor ratio calculations.

The SNP loci were also analyzed here to determine if they present a more accurate representation of mixture ratio at lower concentrations, as they are shorter in length and more readily amplified. The contributor ratios for the SNP loci were calculated to be 1.787, 8.726, and 21.077 for 1:1, 1:4, and 1:9, respectively. Only three SNP alleles dropped out, one at 1:4 and two at 1:9; however, the contributor ratio values were significantly higher than STRs calculated on either the CE or sequencing platform. Overall, the data showed that the MiSeq® FGx was concordant with the CE, which should facilitate the introduction of sequence data as an additional tool for use in forensic DNA testing.

#### Next Generation Sequencing, Mixture Interpretation, Contributor Ratio





### H98 Seasonal Differences in Soil Chemistry and Biology Impacted by Long-Term Human Decomposition

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**Learning Overview:** After attending this presentation, attendees will gain an understanding of patterns in soil chemistry as well as soil microbial and microfaunal community succession resulting from two-year-long human decomposition studies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving understanding of environmental changes that occur during human decomposition in terrestrial environments and how these changes vary seasonally, introducing new indicator organisms, and discussing implications for time-since-death predictions.

Human decomposition creates dynamic biogeochemical hotspots in the soil, resulting in rapid and persistent changes in soil chemistry and biology.<sup>1</sup> Existing studies have largely focused on the period associated with rapid soft tissue loss (i.e., active decay) rather than later time periods; thus, it is not well known to what extent these chemical and biological changes persist. Additionally, little attention has been focused on the impacts on microfaunal members of the soil food web; nematodes in particular are sensitive to environmental disturbances, and they have been proposed as environmental indicators.

The purpose of this study was to perform a long-term, high temporal resolution assessment of changes in soil chemistry in conjunction with microbial and nematode succession beneath decomposing human bodies in order to identify patterns associated with decomposition progression. Six donated human subjects were decomposed on soil at the University of Tennessee Anthropology Research Facility, three in the spring, and three in the winter. Soil sampling consisted of 1cm interfaces and 0–15cm cores from beneath donors. Soil temperature, pH, Electrical Conductivity (EC), Dissolved Oxygen (DO), respiration (CO<sub>2</sub>), NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and extracellular enzymes were measured. Nematodes were counted and identified to family and genus level by microscopy.

In both seasonal studies, the introduction of decomposition fluids into the soil was immediately accompanied by lowered pH and elevated NH<sub>4</sub><sup>+</sup>-N and CO<sub>2</sub>. Interface (0–1cm) samples exhibited the greatest changes, the timing and magnitude of which varied by season. The spring study was marked by the onset of active decay (day 8), accompanied by an immediate increase in EC (mean 969µS cm<sup>-1</sup>). DO decreased 61% by day 12 during late active decay, concomitant with a peak in NH<sub>4</sub><sup>+</sup>-N (mean 6,905µg gdw<sup>-1</sup>). Respiration peaked on day 27 during early advanced decay (mean 10,134ppm CO<sub>2</sub> day<sup>-1</sup>). Unexpectedly, nematode abundances remained suppressed until day 58, after which a bloom of bacterial-feeding nematodes was observed (Rhabditidae). Nematode abundances peaked on day 86 at 29,708 nematodes per 100gdw soil. By the end of the one-year study, the disturbance was still evident: no metrics except DO had returned to control values, and nematode community composition remained shifted toward a predominance of bacterial-feeding taxa.

In contrast, donors in the winter study were marked by a lack of bloating and insect activity due to cooler temperatures. By day 55, EC, CO<sub>2</sub>, and NH<sub>4</sub><sup>+</sup> peaked at mean values of 926.8µS cm<sup>-1</sup>, 9,263ppm day<sup>-1</sup>, and 2,811µg gdw<sup>-1</sup>, respectively. DO decreased by only 29% by day 75, concomitant with a peak in nematode abundance of 116,248 nematodes per 100gdw soil. Nematode abundances began to increase immediately upon deposition of decomposition products into the soil, and communities were shifted toward both opportunistic bacterial and fungal feeders (Rhabditidae, *Aphelenchoides*).

Taken together, these results show that soils continued to be impacted by decomposition products after a year, and that soil microfauna provide valuable supporting information about the decomposition environment.

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#### Reference(s):

1. Keenan, S.W., Emmons, A.L., Taylor, L.S., Phillips, G., Mason, A.R., Mundorff, A.Z., Bernard, E.C., Dovoren, J., DeBruyn, J.M. (2018). Spatial Impacts of a Multi-Individual Grave on Microbial and Microfaunal Communities and Soil Biogeochemistry. *PLoS ONE*. 13(12):e0208845.

#### Human Decomposition, Nematodes, Soil Chemistry



### H99 Jay Dix Memorial Bonus Day

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**Learning Overview:** After attending this presentation, attendees can expect to understand how and why deaths related to the topics specified below occur. Attendees will learn a systematic approach to the evaluation of such deaths that can easily be implemented in their daily practices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a comprehensive review of what causes and contributes to deaths related to the topics specified below. Attendees will be able to systematically evaluate deaths they encounter in their daily practices in which the topics specified below may have played a role.

A proper medicolegal death investigation is a multidisciplinary process that often involves non-medical personnel as well as medical professionals. This annual lecture series provides non-forensic pathologist forensic scientists a basic review of selected topics in forensic pathology in order to increase familiarity and understanding and enhance inter-discipline communication.

This year's lecturers will discuss the medicolegal investigation of deaths related to environmental conditions, deaths due to asphyxia, deaths due to firearm injuries, deaths involving blunt and sharp force trauma, and deaths temporally associated with law enforcement custody.

Electricity is a ubiquitous entity in our lives. Some of it is intentionally generated to provide power, and some of it originates as a force of nature (lightning). Interaction between humans and electricity is common and typically has no untoward effects. However, under some conditions this interaction may result in morbidity and/or mortality. Multiple causes, mechanisms, and contributory factors play a role in injury and deaths involving electricity. Understanding and evaluating injuries and deaths in which electricity may have played a role requires basic knowledge of electricity and how it affects various biological vital functions. Recognition of injuries and deaths caused by electricity is particularly important because of implications regarding the safety of others. This lecture will provide a comprehensive review of these issues.

Blunt and sharp force injuries encompass the major categories of mechanical injury. Blunt force injuries are among the most common injuries sustained by persons. These injuries include abrasions (scrapes), contusions (bruises), and lacerations (tears). Blunt force is also a substantial component of chop wounds, injuries caused by relatively heavy-edged objects, such as a machete or axe. Sharp force injuries result from the mechanical division of tissues by edged or pointed objects. Sharp force injuries include stab wounds, cuts (incised wounds), and chop wounds. Multiple factors and mechanisms are involved in injuries and deaths involving blunt and sharp forces. Understanding and evaluating injuries and deaths in which these forces may have played a role requires basic knowledge of these injuries and how to distinguish them from other types of trauma, recognition of patterned injuries, and recognition of injury patterns. This lecture will provide a comprehensive review of these issues.

Human life requires the uptake and utilization of oxygen along with the release of metabolic waste. Failure of these processes leads to asphyxia. Proper evaluation of asphyxial deaths requires knowledge of the entities that cause asphyxia, pathophysiologic mechanisms, asphyxia death scenarios, and factors that contribute to death. This lecture provides comprehensive discussion of the investigation of deaths that may involve asphyxia.

Firearm fatalities are a major cause of non-accidental morbidity and mortality in the United States. Multiple factors and mechanisms are involved in producing firearm injuries. Understanding and evaluating firearm injuries requires a basic understanding of how these injuries are produced and how to distinguish them from other types of trauma. This lecture will provide a comprehensive review of these issues.

There are multiple causes, mechanisms, and contributory factors that can play a role in deaths that are temporally related to law enforcement apprehension and custody. The apprehension and custody process can be divided into several stages—pre-apprehension, apprehension, and incarceration. Particular diseases and injuries tend to occur and/or become manifest during each of these stages. This lecture will systematically review what diseases and injuries cause/contribute to death in the apprehension and custody process, how they affect physiology and anatomy, when they are typically operative and how they are manifested. This lecture will review the conceptual and practical aspects of understanding and investigating deaths temporally related to apprehension and custody.

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### Medicolegal, Death Investigation, Forensic Pathology

### H100 Volatile Substances Concentrations in Costal Cartilage in Relation to Blood and Urine

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**Learning Overview:** After attending this presentation, attendees will have learned to use costal cartilage to detect volatile substances, such as ethyl alcohol, isopropyl alcohol, and acetone, when soft tissue is not available for analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a method to detect volatile substances with non-soft tissues.

In Poland, there are rare cases of poisoning with non-consumable alcohol. In the material collected during autopsies in cases of poisoning with non-consumable alcohols (i.e., other than ethanol), isopropanol and acetone can be detected. In some forensic autopsies, blood is not available; hence, other matrices are sampled for toxicologic analysis. The goals of the present study were to: (1) examine whether volatile substances, such as ethyl alcohol, isopropyl alcohol, and acetone, can be detected in costal cartilage; and (2) investigate whether analyses of different forms of costal cartilage can give useful information about volatile substance concentrations in the peripheral blood. Presented here are the results of a comparative study of volatile substances concentration in postmortem Costal Cartilage (CC), blood, and urine samples collected during medicolegal autopsies. Examination of this type of material may be useful in cases: (1) when soft tissues are not available or in a state of advanced cadaver decomposition (i.e., as an alternative material); and (2) when the remains are almost completely skeletonized (as the main material).

Ethanol concentrations were determined in samples of Unground Costal Cartilage (UCC), Ground Costal Cartilage (GCC), Femoral Venous Blood (FVB), and Urine (U). UCC was obtained by scalpel fragmentation, whereas GCC was obtained by grinding in 3min preincubation, one grinding cycle for 2min, 12 Cycles Per Second (CPS). The studied group included CCs taken from cadavers in which the presence of ethyl alcohol in blood and urine was demonstrated. The control group consisted of CCs taken from cadavers with no ethyl alcohol detected. The samples were analyzed in duplicate by Gas Chromatography (GC) with a Flame Ionization Detector (FID) using the headspace analysis. The chromatographic separation was performed with a column. T-butyl alcohol was used as an internal standard. Results were obtained for 12 samples.

Distribution of variables was evaluated by the Shapiro-Wilk tests and quantile-quantile plot. The interval data was expressed as a mean value  $\pm$  standard deviation in the case of normal distribution or as a median (lower–upper quartiles; Me (Q1;Q3)) in the case of skewed or non-normal data distribution. Statistical significance was set at a p value below 0.05, and all tests were two tailed. Statistical analysis was performed using a statistics program.

There was a relationship between the method analyzing the amount of ethanol in the urine and the UCC and the GCC methods and the concentration of alcohol in the blood. In all cases, there was a strong positive correlation between the analyzed method and the concentration of ethyl alcohol in the blood (U:  $r=0.899$ ,  $p<0.001$ ; UCC:  $r=0.809$ ,  $p<0.01$ , and GCC:  $r=0.749$ ,  $p<0.01$ , respectively) In addition, there was a relationship between the method analyzing the amount of isopropanol in the urine and the UCC and the GCC methods and the concentration of alcohol in the blood. In all cases, there was a strong positive correlation between analyzed methods and the concentration of alcohol in the blood (U:  $r=0.979$ ,  $p<0.001$ ; UCC:  $r=0.866$ ,  $p<0.001$ ; GCC:  $r=0.942$ ,  $p<0.001$ ). Regarding a relationship between the analyzing acetone concentration methods, the statistical significance was observed only in the case of urine concentration ( $r=0.960$ ,  $p<0.001$ )

Overall, this study showed that higher volatile substance concentrations were determined in ground samples. This study is believed to be novel and the first to demonstrate the possibility of volatile substances detection in the postmortem costal cartilage.

#### Costal Cartilage, Volatile Substances, Gas Chromatography



### H101 The Relationship of Chronic Psychostimulant Use and Cardiovascular Disease

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**Learning Overview:** The goal of this presentation is to assess the relationship between chronic psychostimulant abuse and the development of cardiovascular pathology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by using a retrospective and prospective study to compare cardiovascular disease development in psychostimulant abusers. Cardiac parameters, histological changes, and user demographics are discussed.

There is considerable literature on the contribution of chronic stimulant abuse in causing or contributing to the development of cardiovascular disease. While it seems clear that cocaine and methamphetamine adversely affect the heart and blood vessels, the explanation of how chronic use contributes to cardiovascular disease remains elusive. The challenge in defining their role is because the same diseases are common natural diseases, and it is difficult to assess whether stimulants directly contribute to the development of cardiovascular disease, accelerate it, or are merely coincidental. The present study addresses these questions.

**Methods and Materials:** Using autopsy material from the King County Medical Examiner's Office (KCMEO) in Seattle, WA, this presentation uses two different approaches to assess the role of stimulant abuse in developing cardiovascular disease. The first is a retrospective review of specific cardiac parameters: heart weight and coronary atherosclerosis in decedents of drug overdose involving cocaine, methamphetamine, or heroin. These parameters were compared between two groups: those dying of overdoses including stimulants, with or without heroin, and those dying of overdoses including heroin, without stimulants.

The second approach is a prospective study examining additional cardiac parameters: heart weight, left ventricular wall thickness, coronary atherosclerosis, and cardiac fiber diameter measured histomorphometrically. These parameters were compared between two groups: decedents between the ages of 19 and 39 years dying of causes other than drug overdose who were positive for either cocaine or methamphetamine and decedents in the same age range dying of causes other than overdose who were negative for cocaine or methamphetamine.

**Results:** In the retrospective study, there were 249 decedents positive for methamphetamine or cocaine, with or without heroin. In the stimulant-negative comparison group, there were 193 decedents positive for heroin but negative for cocaine or methamphetamine. The average age was 44 years in the former group and 39 years in the latter group. In the stimulant-positive group, mean heart weight was 554 grams, compared with 425 grams in the stimulant-negative group. Coronary atherosclerosis was present in 37% of the stimulant positive group compared with 26% in the stimulant-negative group.

In the prospective study, there were 28 decedents positive for stimulants and 32 decedents negative for stimulants. The stimulant-positive group had no differences in heart weight and left ventricular thickness, compared to the stimulant-negative group. In the stimulant-positive group, cardiac fiber diameter was statistically greater than that of the stimulant-negative group. In the stimulant-positive group, 32% had coronary atherosclerosis, compared to 6% in the stimulant-negative group.

**Discussion:** The present study uses two novel approaches to further understand the contribution of stimulant abuse toward developing cardiovascular disease. One approach uses a comparison group of overdoses involving heroin, in which stimulants are absent, and the second uses histomorphometry to assess early or subtle changes in cardiac morphometry. Despite obvious limitations in using postmortem data to assess natural disease progression, the results of the two approaches, taken together, provide evidence that stimulant abuse may accelerate the progression of natural disease, starting in early adulthood, and may represent an independent risk factor acting additively or synergistically with other recognized risk factors in promoting the development of premature cardiovascular disease. Furthermore, cardiovascular disease likely represents an important condition predisposing to death due to acute intoxication involving stimulants.

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**Psychostimulant, Cardiovascular, Cocaine**



### H102 Suicide by Acute Substance Intoxication: A Retrospective Analysis of Cases in Cook County, Illinois

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**Learning Overview:** After attending this presentation, attendees will have a better knowledge regarding suicides due to drug toxicity and the features that can help discern them from accidental suicides.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting relevant information related to suicides by drug toxicity in a large metropolitan area to avoid misclassifying the manner of death.

Acute drug toxicity is one of the leading causes of death in the United States. According to the Centers for Disease Control and Prevention, since 2008, suicide has ranked as the tenth leading cause of death for all ages in the United States. Suicide is a cause of preventable mortality, but its prevention requires complex coordination and cooperation among health care providers, individuals and family members, and treatment services and the community. Medical examiners and coroners contribute to suicide prevention by assessing the manner of death. Accurate classification of the manner of death (natural, accident, suicide, homicide, or undetermined) is critical to public health. Information regarding the manner of death is directly reported into a mortality surveillance system that drives prevention, research, policy, monitoring and evaluation, and allocation of resources.

Fatal drug intoxications are thought to be among the most challenging cases for which to determine the manner of death. The intent of an overdose death can be hard to ascertain because of the lack of specific clues or the presence of potentially equivocal evidence regarding intent to die. These deaths are often classified as accidental or undetermined, leading to a potential underestimation of suicides. As a result of this, there is an inaccurate transmission of data to the public health databases. Possible misclassifications adversely impact suicide mortality surveillance, etiologic understanding, prevention, and hence, clinical/public health policy formation and practice.

In the present study, case files from the electronic database of the Cook County Medical Examiner's Office in Chicago, IL, were retrospectively reviewed over a five-year period from August 2014 to August 2019 for suicides in which the primary cause of death was acute drug and/or alcohol intoxication. The following keywords were searched in the "primary cause of death" field: "alcohol," "combined," "drug," "intoxication," and "toxicity." No limits regarding age, race, and sex were imposed. Only cases in which investigative and toxicologic reports were available were included in the study.

A total of 277 cases of suicides meeting the study criteria were found in the office database. The ages ranged from 15 to 91 years (mean: 46 years). There were 130 males, and 147 females. 235 subjects were Caucasian, 30 were African American, 9 were Asian, and 3 were Others. A full postmortem examination was performed in 236 cases.

There is an increasing trend of deaths related to substance overdose in the United States. A careful evaluation of these deaths is mandatory to identify potential suicides, providing a positive impact on public health surveillance and prevention efforts. This study adds valuable information that can be used to help determine the correct manner of death in cases of fatal drug/alcohol intoxication. The results of this study, including demographic comparisons, investigative evidence and toxicological data, will be presented to the attendees.

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#### Suicide, Acute Toxicity, Drugs



### H103 Active Duty United States Military Deaths Due to 1,1-Difluoroethane Intoxication

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**Learning Overview:** After attending this presentation, attendees will understand the prevalence of active duty military deaths due to intentional inhalation of difluoroethane and discuss possible preventative measures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing that all forensic autopsies performed should have routine testing for difluoroethane, especially in circumstances suggestive of its use, such as the presence of numerous cans of compressed air on scene.

Huffing, sniffing, or bagging of compressed air is a common method to “get high,” especially in teenagers and young adults, due to ease of access and difficulty in detecting its abuse. Compressed air contains 1,1-difluoroethane, a halogenated hydrocarbon gas; intentional inhalation can result in central nervous depression and cardiotoxicity, including sudden death, which is believed to be due to cardiac arrhythmias. Routine workforce drug screening, including the Department of Defense (DoD) Drug Testing Program, does not currently screen for volatiles such as difluoroethane; thus, abuse can go undetected (although some military services do require a breathalyzer test at the time of urinalysis to detect the presence of ethanol).

A retrospective review of all autopsies performed by the Armed Forces Medical Examiner System (AFMES) from 2010 until 2018 revealed 20 active duty service member deaths that were directly attributed to 1,1-difluoroethane intoxication. Four deaths of civilians (either military dependents or federal employees) due to difluoroethane were also noted but are not discussed further in this abstract. Scene investigation typically revealed numerous cans of compressed air. Autopsy findings were non-specific, and the most common finding was pulmonary edema. Although frostbite on the hands and face has been reported in the literature, this finding was not documented in any case. Histology was performed in about one-third of cases and again showed non-specific findings, with pulmonary edema the most common histology finding. Specimens submitted for toxicology testing (if able to be obtained) included peripheral blood (submitted in gray, purple, and red top blood tests), urine, vitreous fluid, gastric contents, bile, brain, heart, lung, liver, spleen, kidney, and adipose tissue. No special handling or unusual specimens, such as tracheal aspirates, were required for testing. Full postmortem toxicological testing was performed in all cases to include analysis via headspace gas chromatography with a flame ionization detector for volatiles. All positive findings were confirmed on at least two different bodily fluids and/or tissues using headspace gas chromatography/mass spectrometry.

A query of AFMES’ mortality data revealed an additional 12 deaths certified as difluoroethane intoxication by civilian jurisdictions over the same time period. Autopsy findings, other than cause and manner of death, and toxicology testing results were not available for the 12 cases performed by civilian jurisdictions. Demographics of all 32 decedents included predominantly men (91%), with an age range of 20 to 44 year (average age: 30 years). The decedents served in all branches of the United States Armed Forces and were predominantly in the enlisted ranks, although two officer deaths were noted.

Several years ago when difluoroethane deaths became more recognized in the United States, bitterants were added to the cans of compressed air, with the goal of reducing abuse. This office recommended that all cans of compressed air available at military shopping facilities have the bitterant. However, this data shows that deaths due to difluoroethane continue to be detected. Clearly, inhalant abuse is significantly underrecognized in the public and in active duty service members. This study identified 32 deaths of active duty service members over the past ten years that possibly could have been prevented if antemortem abuse was detected.

#### **Huffing, Difluoroethane, Military**



### H104 Why the “Kontroversy”? Is Kratom a Killer? The Emergence of Mitragynine in Drug-Associated Deaths in West Tennessee

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**Learning Overview:** After attending this presentation, attendees will have: (1) become familiar with the mechanism of action, adverse effects, and the legal status of kratom; (2) examined the epidemiology of kratom users; and (3) studied the relationship between mitragynine and other drugs on death certificates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness that kratom, the herbal medicinal plant native to Southeast Asia, with the major alkaloid mitragynine, continues to be increasingly implicated in drug toxicity-related deaths, either in multidrug toxicity and, rarely, as a sole cause of toxicity. Understanding the patterns of kratom use and associated drugs may be of use for the formation of public policy regarding this drug on the state and federal levels.

*Mitragyna speciosa* is a tropical evergreen tree that is a member of the coffee family, and is native to Southeast Asia, including Thailand, Malaysia, and Indonesia. The plant is commonly called “kratom” in Thailand. The leaves of the plant have been used in traditional herbal medicine for decades by being chewed, smoked, or brewed into a tea. The leaves contain over 25 various natural alkaloids, including the main indole alkaloid mitragynine, which reportedly accounts for approximately 66% of the alkaloid content. Mitragynine is an agonist of the  $\mu$ -,  $\kappa$ -, and  $\delta$ -opioid receptors; however, it is unique in that it has stimulatory effects at low doses and behaves as a sedative at higher concentrations. The controversial kratom has been shown to have addictive properties, and withdrawal symptoms have been reported anecdotally and in literature. The Drug Enforcement Administration (DEA) briefly added kratom to its list of Schedule I drugs in August 2016, but reversed its decision in October 2016 due to a large public outcry and currently the DEA classifies kratom as a “Drug and Chemical of Concern.”

All autopsy records and toxicology records (from the National Medical Services [NMS] laboratory) occurring in the jurisdiction of the West Tennessee Regional Forensic Center from 2015–2019 were reviewed for mitragynine. Mitragynine began to emerge at the West Tennessee Regional Forensic Center in 2016, with three positive cases. No cases were detected in 2017, and seven cases were detected in 2018. Three cases have occurred in 2019 thus far.

Of the 13 deaths that had mitragynine detected on postmortem toxicology, 11/13 (85%) were male, 13/13 (100%) were White, and the median age at the time of death was 32 years (range 22–54). In 12/13 (92%) of these deaths, mitragynine was listed on the death certificate in combination with other drugs. One death could not determine the degree mitragynine contributed, and was therefore not listed on the death certificate. In one death, mitragynine was listed as the sole cause of death in part 1, along with coronary artery disease and chronic alcoholism as contributing conditions.

A quantifiable mitragynine level was available in 10/13 (77%) of cases (NMS laboratory did not quantify mitragynine until 2017; thus, in the remaining three cases, the level was qualitative only). Of quantified levels, the median mitragynine level was 73.5ng/mL (range 25–680ng/mL). When multidrug toxicity was involved, drugs on the death certificate included fentanyl (6/12, 50%), alprazolam (6/12, 50%), ethanol (4/12, 33%), morphine (4/12, 33%), heroin (3/12, 25%), and 7-amino clonazepam (3/12, 25%).

This study demonstrates that mitragynine emerged in West Tennessee around 2016 and has continued to increasingly appear in deaths in West Tennessee, which is in concordance with NMS laboratory data. Interestingly, 2017 had no deaths with positive postmortem results for mitragynine. 2018 had the majority of the mitragynine-associated deaths, and already three deaths have been seen in association with it in 2019 (as of July 2019). A majority of the affected decedents were White males with a median age of 32 years. When mitragynine was seen in conjunction with multidrug toxicity, other opioids and central nervous system depressants were the most commonly seen, including fentanyl and alprazolam. Based on the high numbers of mitragynine in conjunction with fentanyl, perhaps the mitragynine powder is used as a bulking agent for fentanyl. Rarely, mitragynine is seen as a sole cause of death. Mitragynine continues to be detected on postmortem toxicology across the nation, and this study is presented to help further educate and help form future policy regarding kratom and to also prevent further deaths.

#### Kratom, *Mitragyna speciosa*, Toxicology

## H105 Pediatric Poisonings: An Epidemiological Study

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of childhood poisonings and of designing a detailed report to alert and inform the authorities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees about the clinical forensic medicine applications, examinations, and reporting of poisonings in a standard base so all medical doctors may improve their services with better understanding.

Poisoning is one of the most common medical emergencies in childhood. Poisoning is defined as cells being injured or destroyed by inhalation, ingestion, injection, or absorption of a toxic substance.<sup>1</sup> The prevalence and type of poisoning varies worldwide depending on the particular topography/environment, type of agricultural activities, amount of industrial development, cultural practices, and local beliefs/customs. The case fatality rate is largely determined by the degree of access to quality health care treatment.<sup>2</sup> Although pediatric poisonings are considered emergencies, more than 85% of cases need no medical intervention because the ingested material is typically not toxic or the amount swallowed is not clinically significant<sup>1</sup>.

Each year in the United States, more than one million poison exposures among children younger than six years of age are reported to the American Association of Poison Control Centers (AAPCC).<sup>1,3</sup> According to the World Health Organization (WHO), in 2004, acute poisoning caused more than 45,000 deaths in children and teenagers.<sup>3</sup> The most common agents implicated in childhood poisonings in developed nations were over-the-counter preparations (e.g., paracetamol, cough/cold remedies, vitamins and iron tablets, antihistamines, and anti-inflammatory drugs); prescription medications (e.g., antidepressants, narcotics, analgesics); recreational drugs; household products (e.g., bleach, disinfectants, detergents); pesticides (e.g., insecticides, rodenticides, herbicides); poisonous plants, and animal or insect bites.<sup>1</sup>

This is a retrospective study. Included in this study are all poisoned children admitted to the Forensic Medicine Council of Adana/Turkey in 2018. Data were collected from the medical records of children  $\leq 18$  years of age. In sum, acute poisoning was reported in 108 children. Of the total acute poisoning cases, 46.2% were males and 53.7% were females. Of the 108 cases, the youngest age was eight months and the average age was 78.4 months. Pharmaceutical agents were identified in 57.4% of cases. Analgesics were the most frequently ingested drugs (23.1%). Therapeutic drugs were most common cause at all ages. The manner of death in most of the drug intoxication cases in Turkey is suicide in adults; however, it is mostly accidental in childhood cases.<sup>4</sup> In the current study, accidental drug poisonings comprised 64.8% of total poisoning cases, while suicides accounted for 35.1%. Non-pharmaceutical agents were identified in 46 patients (42.5%), 20.3% of which were food poisoning. Other intoxications (24 cases; 22.2%) ordered by frequency were with mushrooms (9.2%), organophosphates (5.5%), carbon monoxide (4.6%), and others (2.7%).

Acute poisoning is considered as one of the most common medical emergencies in children.<sup>1,3</sup> Accidental poisonings are still highly common in most countries, despite the improvement of preventive measures, which consist of proper packaging of medicines and household products, doubled by raising awareness of the toxic items that should be kept out of children's reach.<sup>5</sup> Parental education about the prevention of childhood poisoning at home remains a major issue, especially with regard to keeping everyday medications and household products out of the children's reach. The use of child-resistant containers for medications or household products and the use of locked cabinets would help in prevention efforts.<sup>6</sup>

This study provides significant demographic data that can assist in alerting clinical colleagues to take further precautions. Therapeutic drug intoxications are one of the major serious childhood causes of admission to emergency services; patients typically recover much better with an appropriate treatment and follow-up. Forensic clinical examinations are important in daily practice as they can provide detailed reports informing clinicians—as well as emergency service personnel and Intensive Care Unit (ICU) caretakers—about childhood poisoning epidemiologic factors and risks.

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### Pediatric Poisonings, Accidental, Clinical Forensic Medicine





### H106 An Opioid Analysis in “Natural” Manner Scene Inspection Cases

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**Learning Overview:** The goal of this presentation is to report a study investigating the increase in drug intoxication deaths in the older population and the results of performing drug analysis on natural manner scene inspection-only cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the need for thorough scene inspections in supposed natural manner cases.

At the State of Maryland Office of the Chief Medical Examiner (OCME), the majority of cases that fall under Medical Examiner (ME) jurisdiction are autopsied, but there can be a scene inspection by investigators with the ME completing the Death Certificate (DC). Typical scene inspections are cases  $\geq 55$  years of age with significant medical history. On non-natural scene inspections, two tubes of blood are drawn at the scene and sent to the OCME for toxicologic analysis. The analysis is primarily for ethanol but can be for other drugs as needed. Blood is not drawn on a natural manner scene inspection case. Since 2010 in the State of Maryland, there has been a five-fold increase in drug intoxication deaths for people over the age of 55 years. In 30%–33% of cases where heroin and fentanyl were positive, the decedents were 50 to 70 years old. Since many individuals  $\geq 55$  years of age fall under scene inspection criteria and since the age of these autopsied opioid deaths was increasing, this study investigated whether there were missing opioid deaths in this age group.

During a five-month period (163 days) from 4/8/2018 to 1/18/2019, the OCME performed a study looking for opioids/fentanyl in natural manner scene inspections in two of their highest volume counties, Baltimore County (BC) and Prince Georges County (PGC). Investigators drew blood and blood specimens that were screened for volatiles, morphine, and fentanyl. Volatiles screening for ethanol, methanol, acetone, and isopropanol was performed by headspace gas chromatography. Morphine screening was performed by Enzyme-Linked Immuno-Sorbent Assay (ELISA) with a cutoff concentration of 10ng/mL. Positive morphine ELISA results were confirmed by gas chromatography/mass spectrometry with a 10ng/mL reporting limit. Fentanyl screening was performed by gas chromatography/mass spectrometry with a 1.0ng/mL reporting limit.

During the study period, there were 400 total scene inspection cases in the state. There were 39 total scene inspections for BC and 87 for PGC. Of those, there were 13 non-natural cases in BC and 8 non-natural cases in PGC. All were suicides. This left 26 total “natural” scene inspections in BC and 79 in PGC. In 47 (45%) of these 105 cases, no toxicology sample was obtained for various reasons.

A total of 58 cases had toxicology drawn and only one (1.7%) tested positive for opioids. The age range was 40 to 93 years, mean age 68.67 years; 31% were female and 69% were male; 57% were African American, 31% were Caucasian, 8% were Hispanic, 2% were Asian, and 2% were Other. The positive case was a 59-year-old African American male from PGC with no Maryland physician, a medical history of hypertension, heart disease status/post (s/p) Coronary Artery Bypass Graft (CABG) and aortic valve replacement, Cerebrovascular Accident (CVA), and Gastroesophageal Reflux Disease (GERD) found unresponsive at home. There was no history of drug use reported and no illegal drugs or drug paraphernalia were present at the residence. Toxicologic analysis of heart blood showed fentanyl (0.010mg/L), free morphine (56ng/mL), and unquantified despropionyl fentanyl. His Death Certificate (DC) had been signed as Heart Disease with Aortic Valve Replacement, Natural. The DC was amended, adding the drug use and changing the manner to Undetermined.

In conclusion, a thorough scene inspection is necessary to make the appropriate decision to bring a case in for autopsy. Only one case in this study was positive for opioids. In a five-month period in these two high-volume counties, one opioid overdose case that would normally have been left out as a scene inspection and signed out as natural would have been missed. The study group is too small to be statistically relevant; however, it appears that if thorough scene investigation protocols are followed, the chance of missing an opioid death in an older individual with health history is small. The investigators are only as good as the information provided to them and their personal evaluation of the scene. In the future, the only way to catch this type of case would be to change the scene inspection protocol to include drug testing on all suspected natural deaths or to bring these cases in for autopsy. The physical and financial burden placed on investigators, toxicology staff, and medical examiners appears to outweigh the benefit of catching such a small percentage of cases.

#### Fentanyl, Opioid, Inspection



## H107 Fatal Excipient Lung Disease: An Autopsy Case Series

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**Learning Overview:** The goal of this presentation is to delineate the spectrum of clinical presentations and autopsy findings encountered in patients with fatal excipient lung disease and the need for careful scrutiny of deaths in patients receiving prescribed opioids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a non-specific spectrum of gross, histologic, and circumstantial findings that are associated with excipient lung disease, further decreasing the misclassification and underreporting of this entity.

**Introduction:** Excipients are described as filler or binder materials found in oral tablets or pharmaceutical substances. These materials are inactive, inert, insoluble, and added to pharmaceutical compounds to enhance the lubrication effect, form, absorption, solubility, and/or shape of the tablet. Some excipients found in oral tablets include talc (magnesium silicate), magnesium stearate, fumed silica, microcrystalline cellulose, crospovidone (a form of Polyvinylpyrrolidone [PVP]), and starch. Excipients and adulterants similar to those listed may be found in illicit drugs, such as heroin formulations representing typical contaminants or dilutants.

When pharmaceutical-grade tablets are crushed and injected intravenously, the excipient may induce a foreign body angio-granulomatous reaction within the lung, known as “excipient lung disease.” This foreign body granulomatous reaction is typically histopathologically similar to the described reactions in illicit intravenous drug user injection. Clinically, the patient can present with a toxidrome, as well as acute, subacute, or chronic signs and symptoms, such as sudden or unexpected death, pain, dyspnea, fever, pulmonary fibrosis, acute lung injury, thromboembolism, pulmonary hypertension, and/or non-specific centrilobular nodularity on imaging. As this medication may be prescribed to the patient for a legitimate medical reason or diagnosis, in the setting of positive detection by toxicologic studies, the pulmonary findings can be overlooked or attributed to another disease process and, therefore, left unchecked, resulting in respiratory failure. Identification of this pathologic process at autopsy is usually not difficult; however, attributing the disease to excipients and the correlation with the toxicology and circumstances can make the cause of death determination challenging. Furthermore, lack of recognition by clinicians and radiologists will leave many cases of excipient lung disease underreported and, therefore, misclassified as frequently the injection of the crushed tablets is clandestine. Reported herein is the autopsy findings in three cases of excipient lung disease in patients with complex medical histories and a range of clinical presentations.

**Methods:** Three cases from the Miami-Dade Medical Examiner and Las Vegas Clark County Coroner were identified during the period of 2015–2018. Autopsy reports and case files were reviewed, and pertinent gross and histologic findings were tabulated.

**Results:** All toxicology levels were either within therapeutic range and/or not considered fatal.

	Age; Sex	Medical Conditions	Known Intravenous Drug Use	Clinical Diagnostic Suspicion	Polarizable Foreign Material in Lungs	Postmortem Toxicology	Cause of Death
1	22; F	Sickle Cell Anemia, Depression, Opioid Dependence	No	None	Large Quantity	Oxycodone	Excipient Lung Disease
2	28 F	Asthma, Substance Abuse, Endocarditis, Arm Deep Vein Thrombosis	Yes	Mycobacterial Infection	Large quantity With Granulomas	Oxycodone, Quetiapine, and Venlafaxine	Excipient Lung Disease
3	44; F	Bipolar Disorder, Asthma, Obesity, Crohn’s and History of Hodgkin Lymphoma	No	Atypical Mycobacterial Infection	Large Quantity With Granulomas And Fibrosis	Fentanyl, Codeine, and Promethazine	Excipient Lung Disease

**Conclusion:** Excipient lung disease can be a difficult clinical and radiologic diagnosis due to the non-specific diagnostic findings and the range of clinical presentations along with potentially complex competing diagnoses. All three patients were prescribed opiates for underlying medical conditions. Both typical and atypical mycobacterial infection were considered in two of the three cases as the probable cause of the underlying lung disease. The cases described herein further delineate the potential spectrum of clinical presentations and autopsy findings encountered in patients with fatal excipient lung disease and the need for careful scrutiny of deaths in patients receiving prescribed opioids.

### Excipient, Lung, Angiogrammatous Inflammation

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### H108 Loperamide Abuse: A Rising Public Health Concern?

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**Learning Overview:** After attending this presentation, attendees will better understand the pharmacology and toxicology of loperamide and its rising popularity as an opioid drug of abuse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a case report of a young woman who died of loperamide toxicity and by allowing for increased recognition of this potential drug of abuse that is readily available over the counter.

**Introduction:** Loperamide is a well-known, cheap, anti-diarrheal medication with  $\mu$ -opioid agonist activity that has been available over the counter in the United States since 1988.<sup>1</sup> Loperamide achieves an anti-diarrheal effect through binding opioid receptors in the wall of the bowel and inhibiting the release of chemical mediators, such as prostaglandins and acetylcholine, which leads to a reduction in peristalsis activity.<sup>2</sup> In addition, loperamide is thought to block calcium channels, which also contributes to a decrease in gastrointestinal motility.<sup>2</sup> This medication was originally thought to have little misuse potential for several reasons. These reasons include poor oral bioavailability, rapid hepatic first pass metabolism by the cytochrome P450 complex, and p-glycoprotein mediated efflux out of the central nervous system.<sup>3</sup> However, an opioid toxidrome, including euphoria, has been reported when taking large doses of up to 50–100 2mg pills.<sup>3</sup>

**Materials and Methods:** The decedent was a 27-year-old woman with chronic pain due to severe interstitial cystitis that was being treated with a sacral neurostimulator. She was found by her live-in boyfriend after complaining of nausea and vomiting throughout the day. Based on prescribed medication at the scene and medical records, the decedent may have had an opiate abuse or dependence problem.

**Results:** At autopsy, there was no evidence of significant trauma. Toxicology results revealed the decedent was positive for loperamide at levels of 630ng/ml and diphenhydramine at 2,000ng/ml. A single standard 2mg dose of loperamide gives an average peak plasma loperamide level of 0.24ng/ml at approximately six hours.<sup>5</sup> The toxicology results indicated the decedent took a dose of loperamide at orders of magnitude higher than the recommended dose. The amount of diphenhydramine was also unusually high. It is unknown if this drug played any role in potentiating the effect of the loperamide. The cause of death was determined to be due to the combined toxic effects of loperamide, diphenhydramine, and dextromethorphan.

**Conclusion:** This case report illustrates the potential toxic effects of loperamide when ingested in quantities that are far above the recommended dosing levels. Documented adverse effects from loperamide overdose include fatal cardiac arrhythmia, loss of consciousness, Electrocardiogram (ECG) alterations, and hypokalemia.<sup>4</sup> The opioid crisis occurring in the United States is undeniable. As greater restrictions are put on traditional opiate medications, addicts will likely increasingly turn to more available alternatives and over-the-counter medications such as loperamide. This has already begun to occur. Internet posts about illicit loperamide use have increased from 50 posts a quarter in 2005 to more than 275 posts per quarter in 2011, with an associated increase in published case reports of ten-fold by 2014.<sup>2,4</sup> Recognition and greater awareness of using these widely available alternative medications for obtaining a high is crucial for the public health.

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#### Loperamide, Overdose, Forensic Pathology

## H109 Body Packing of Narcotics Leading to Gastric Perforation and Death: A Case Report and Review of the Literature

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**Learning Overview:** The goal of this presentation is to address/highlight: (1) forensic investigation into body packing; (2) an unusual presentation of narcotic smuggling causing death; (3) the medical risks posed by body packing; and (4) differential diagnosis of body packing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of autopsies and forensic death investigations by showing an unusual presentation of body packing and by reminding investigators to keep a broad differential. This presentation will also serve to increase the body of knowledge around this activity, as it is most likely currently underreported. Attendees will come away with a greater understanding of narcotic body packing, which will hopefully improve performance in examination of this entity.

Gastric perforation leading to peritonitis can be due to an ulcer, malignancy (e.g., adenocarcinoma, lymphoma, or gastrointestinal stromal tumor), or penetrating trauma (e.g., gunshot or stab wound.) Presented here is a highly unusual and forensically relevant case of gastric perforation leading to organizing peritonitis due to the ingestion of a large number of packets (pellets) containing narcotics. The decedent was a 69-year-old man from Ghana, who prior to his death had only been in the United States for a single day and was found unresponsive in the bathroom of his friend's residence. The decision to perform an autopsy was primarily based on his recent long-distance travel and lack of any other medical history.

On external exam, the decedent was an overweight Black man with a Body mass Index (BMI) of 26.5 and a protuberant abdomen. Postmortem examination revealed organizing peritonitis with tan exudates covering the peritoneal lining serosal surfaces and a perforation in the pre-pyloric region of the stomach. Inside the stomach, there were 28 oblong, tightly wrapped latex packets, each measuring approximately 1½ x ½ inches and containing a white powder. The packages were distending and stretching the stomach; friction had most likely caused the wall of the stomach to rupture. All packages were intact. In addition to the 28 found in the stomach, 2 other packages were found that had progressed to the large bowel. The drugs were photographically documented and submitted as evidence to law enforcement agents.

Femoral blood toxicology was positive for delta-9-tetrahydrocannabinol. No cocaine, heroin, or fentanyl were detected in the decedent's blood. Other significant findings included hypertensive cardiovascular disease and pulmonary emphysema. There was no evidence of a pulmonary embolus. Cause of death was determined to be gastric perforation due to foreign bodies, and the manner was determined to be an accident.

Body packers, also known as "mules," are individuals who swallow or insert into a body cavity packets filled with illegal drugs for the purpose of evading customs. Body packers from poverty-stricken countries such as Jamaica and Colombia are recruited and financially compensated to transport drugs to the United States and Europe. Narcotics are usually packed in capsules, condoms, balloons, plastic bags, or fingers of latex gloves and most frequently are swallowed into the stomach or inserted into the rectum or vagina.<sup>1</sup> There are multiple reports of these packages rupturing within the gastrointestinal tract, leading to acute intoxication of the individual.<sup>2-5</sup> Rupture and acute intoxication are the most common causes of death and the risk of package rupture increases with prolonged flight time.<sup>6,7</sup> Medical examiners and coroners need to have a high index of suspicion in cases of sudden death in individuals who have recently traveled from high drug-traffic countries.

Presented here is a much less frequently encountered complication, that of gastric ulceration and perforation, leading to organizing peritonitis, sepsis, and death in a body packer from Africa. This case was unique not only because the mechanism of death was peritonitis rather than intoxication, but also because this individual was elderly and from a country, Ghana, not usually associated with drug trafficking to the United States. In fact, this individual would not have been autopsied except for the fact that he had recently flown into the United States from Africa just a day prior to his death, which led the medical examiner to perform an autopsy to determine whether his death was due to cardiac causes or a pulmonary embolus. The presence of the drugs and his cause of death were both surprising, and somewhat concerning, given that elderly patients that appear to have died of natural causes are not autopsied in many offices around the country. This case illustrates that body packing is used by people of all ages and that without an autopsy, not only is the true cause and manner of death missed, but the incidence of body packing is likely underestimated.

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### Body Packing, Narcotic Smuggling, Drug Mule



### H110 Anticoagulation and Exsanguination: A Case Series of Fatalities From Superficial Wounds in the Elderly

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**Learning Overview:** The goal of this presentation is to bring awareness to attendees that when under anticoagulation treatment, and even within the therapeutic threshold, an elderly person can quickly exsanguinate on a superficial wound. The subsequent manner and cause of death can be difficult to ascertain because the scenes can require forensic analysis due to the amount of blood present.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that superficial wounds can lead to fatal exsanguination in the elderly and present as a crime scene.

There has been an unprecedented anticoagulant use in the United States, particularly in the elderly, including treatment for heart disease, atrial fibrillation, and deep vein thrombosis prophylaxis.<sup>1</sup> Since the advent of Direct Oral Anticoagulants (DOACs), warfarin use has fallen but still accounts for roughly 1.6 million quarterly doctor visits.<sup>2</sup> Warfarin's mechanism of action relies on blocking the vitamin K pathway in the liver and is drastically affected by dietary sources, requiring close monitoring. In contrast, dabigatran and other DOACs employ an anti-platelet pathway that does not interfere with vitamin K or require specific testing or close monitoring. Regardless of the therapy used, patients are at increased risk of bleeding-related complications, even when they are in the therapeutic window of treatment. Emergent reversal of anticoagulation with warfarin employs Fresh Frozen Plasma (FFP) and vitamin K, but is very limited when antiplatelet agents are used.

The aim of this presentation is to bring awareness to the potential lethal adverse effects of anticoagulants and that vulnerable populations can suffer mortal complications due to relatively superficial and minor wounds. This case series of three geriatric patients highlights a rare complication of massive exsanguination from rather subtle injuries consisting of puncture wounds or abrasions less than a one-quarter of an inch. The three patients ranged in age from 85 to 89 years. The corresponding death scenes were often so bloody they required careful forensic evaluation to determine manner and cause of death, including exclusion of foul play. The first decedent, an 85-year-old male, was found deceased at home in a puddle of blood on his kitchen floor. It had been apparent he had made several trips to the bathroom and back and upon external examination, the only wound present was a one-eighth-inch abrasion on his ankle. Another patient, an 89-year-old female, died under similar circumstances after apparently puncturing her ankle on a screw protruding from her walker. The puncture wound was also about one-eighth inch in circumference and the only lesion found on her body. Finally, an 89-year-old male bled out from a one-quarter inch puncture wound on his ankle as well. All three patients were known to be anticoagulated for atrial fibrillation. These findings may become more common as there is increasing prevalence of anticoagulant usage with the aging population. It is important for clinicians to weigh the cost versus benefit of putting a patient on anticoagulants, or these unfortunate deaths due to minor trauma with fatal outcomes will only increase.

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#### Anticoagulation, Exsanguination, Elderly



### H111 2,4-Dinitrophenol Toxicity: A Cause of Death That Is Making a Comeback

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**Learning Overview:** After attending this presentation, attendees will better understand the toxic effects of 2,4-Dinitrophenol (DNP), as well as the antemortem and postmortem evidence associated with its use.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of DNP toxicity and its re-emergence in a culture with increased access to the internet and heightened concerns for body weight and self-image.

DNP is a synthetic chemical that has been used in the production of pesticides, wood preservers, dyes, photographic developers, and explosives.<sup>2-5,7</sup> It acts by uncoupling oxidative phosphorylation, causing the inefficient production of Adenosine Triphosphate (ATP) and increased metabolism of carbohydrates and lipids.<sup>5,7</sup> Because of this, it was introduced into medical practice as a weight-reduction drug in the 1930s, but was later banned due to its potent toxicity and adverse side effects.<sup>2,3,6,7</sup> Despite legislation and warnings of harm associated with its use, reports of deaths due to the use of DNP have increased in the past few years.<sup>2</sup> This may be due to the fact that DNP is readily available online, sold by unregulated vendors and promoted aggressively among the body-building community.<sup>5</sup> There is also increasing concern of usage within vulnerable groups, such as those with eating disorders or young and naïve users who are not fully informed about the drug.<sup>1</sup> The resurgence of DNP-related fatalities could pose a problem for emergency room physicians and medical examiners, as there is no antidote for the compound, and proper diagnosis of DNP poisoning becomes challenging without knowledge of its use.<sup>3,5</sup>

A 47-year-old White male with a past medical history of hypertension was found unresponsive in the bed of his residence by his roommate. The prior night, he experienced fever, labored breathing, and excess sweating. Emergency medical services were contacted, but resuscitative measures were not initiated once postmortem changes were observed. Scene examination revealed a substantial number of stray pills throughout the bedroom and neighboring bathroom. Several prescription medications were located, in addition to vitamins, an empty vial of testosterone, and a used syringe. The decedent had an interest in weightlifting and had a muscular build. Approximately a month after the death, a call was received from the decedent's roommate stating he found evidence to suggest the decedent may have been taking a dietary supplement called DNP at the time of his death. The roommate found email correspondence on the decedent's computer in which he was attempting to purchase the supplement online.

Autopsy findings were significant for moderate pulmonary congestion, focal hemorrhagic gastritis with pill residue in the gastric contents, slight yellow discoloration to the skin of the right arm, and intensely yellow-colored urine. Initial toxicological analysis revealed non-toxic levels of alprazolam, temazepam, oxazepam, lamotrigine, sertraline, and venlafaxine metabolites. After learning of the decedent's potential use of DNP, additional samples were sent to the Center for Forensic Science Research and Education for DNP testing. The samples came back positive for the compound. The cause of death was certified as "2,4-Dinitrophenol Toxicity," with the manner of death being certified as accident.

Given that making a diagnosis of DNP toxicity requires a high index of suspicion and does not present itself in routine toxicological analyses, it is important to understand the factors that may affect an individual's willingness to take this drug, as well as the toxidrome of DNP.<sup>5</sup> This case is presented as an example of how investigative information, autopsy findings, and toxicological analysis must be combined to diagnose DNP toxicity in a deceased individual.

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#### 2,4-Dinitrophenol, DNP, Toxicity



### H112 Death by Intracorporeal Fentanyl Extravasation During the Replacement of an Intrathecal Pump

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**Learning Overview:** After attending this presentation, attendees will be informed regarding an atypical overdose in a patient undergoing chronic therapy with intrathecal administration of fentanyl and the importance of correctly knowing the distribution and metabolism of fentanyl.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the difficulty of determining toxic values of fentanyl in subjects in chronic therapy and the lack of knowledge by clinical physicians regarding the metabolism of fentanyl and its difficulties in therapeutic management.

A 59-year-old woman with type I neurofibromatosis and chronic radiculopathy causing intense pain was treated with intrathecal fentanyl injection via a continuous-release pump implanted in the left abdomen in 2009. During a routine filling of the pump at 1.00 p.m. on December 7, 2017, there was an intracorporeal fentanyl solution extravasation of about 8.7ml (corresponding to 13,050ug of fentanyl). The woman was treated with immediate clinical monitoring and with naloxone therapy (both in bolus and in continuous infusion for anticipated bradypnea). The woman was then hospitalized for 24 hours of monitoring and administration of low doses of naloxone (0.1mg/h) and oxygen therapy. During hospitalization, there were episodes of bradypnea (with an episode below 10 breaths/minute), episodes of hypotension (68/54mmHg), and some periods of desaturation (average saturation 92%–93% with episodic 75% and 89% drops).

In the morning, the woman was visited and appeared well; the administration of naloxone and oxygen was stopped at 9:00 a.m., and the woman was discharged around 12.00 a.m. on December 8. Notably, at discharge a nurse had measured an oxygen saturation of 75% that was not reported to the doctor. For the passage of the woman from the bed to the wheelchair, a mechanical lift was used with belts located in the abdominal region. The woman returned home, reported being particularly tired and sleepy, and went to bed, where she was found dead at about 7:00 p.m. An autopsy was performed and revealed chronic cardiomyopathy, serous pleural effusions, splenomegaly, chronic liver disease, and multiple cutaneous neurofibromas. The pump for intrathecal infusion was positioned in the abdomen and surrounded by a fibrous envelope; transparent liquid was collected between the pump and the casing. Histologic examination confirmed the macroscopic data and showed microscopic alterations due to pulmonary arterial hypertension.

Interpretation of toxicologic results was complicated by multiple factors, including the absence of reference data regarding toxic/lethal fentanyl concentrations in patients chronically medicated via intrathecal administration. The compendium of all available data, however, ultimately led to certification of fentanyl overdose as the cause of the death. Per literature data, a blood concentration of fentanyl of 25µg/l (i.e., the concentration detected in a sample procured around 12 hours after death) was well above therapeutic values and within the range of toxic and/or lethal values. Furthermore, in this case, fentanyl concentrations detected in organs (i.e., brain, liver, and kidneys) were likewise found to fall into the toxic/lethal range. Analysis of samples taken near the pump (i.e., overlying liquid, fibrous tissue, and adipose tissue) likewise showed high concentrations of fentanyl. The highest tissue concentration was recorded in the adipose tissue underlying the pump (9.0mg/l); in sum, these findings would suggest substance redistribution from the extravasation point to the periphery was still in progress at the time of death. The pharmacokinetics of fentanyl in the present case was not comparable to those of intravenous therapeutic or accidental administration of the substance. It is suspected that drug accumulated initially between the pump and the fibrous tissue that covered it, and subsequently progressively distributed to the surrounding adipose tissue and the periphery—a distribution that was still ongoing at the time of death.

The Public Prosecutor then conducted a thorough investigation with reports by the doctors and nurses who treated the woman, highlighting a fundamental ignorance of the notion of a possible reservoir of the fentanyl in adipose tissue with subsequent progressive release into the body (as a lipophilic substance). The anomalous “delayed” release mechanism, not known in practice, has led to the filing of criminal proceedings against the operating medical staff.

#### Fentanyl, Chronic Therapy, Intrathecal Administration



### H113 “Everybody Hurts”: Fatal Anaphylactic Shock Induced by an Intramuscular Injection of Diclofenac

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**Learning Overview:** After attending this presentation, attendees will appreciate forensic methodology for the postmortem diagnosis of anaphylactic shock induced by an intramuscular administration of diclofenac in an adult. The increasingly large use of painkillers worldwide represents an alert for the forensic community for complete postmortem investigations in cases of sudden unexpected death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a case of anaphylactic shock induced by a widely used non-steroidal anti-inflammatory drug. The rarity of fatal events with diclofenac makes this case unusual and interesting for the overall scientific community.

The epidemiological peculiarities of anaphylaxis remain challenging to assess and often the only available data is obtained from hospital admissions. In general, the number of hospitalizations for anaphylaxis is increasing in many countries, with a greater incidence of the phenomenon in the pediatric age group. Prevalence estimates differ worldwide with values varying from 0.3% in Europe to 1.6%–5.1% in the United States. Diclofenac is a widely used Non-Steroidal Anti-Inflammatory Drug (NSAID) derivative of phenylacetic acid. It is commonly used in pain management and preferred by both clinicians and patients because its effect begins in only a few minutes. Safety data from clinical trials in the United States have shown that diclofenac sodium has lower rates of adverse reactions than any of the other comparative NSAIDs. Allergic reactions after intramuscular injection, such as urticaria, asthmatic attack and Kounis syndrome, are commonly reported, while anaphylactic shock is rarely described. A rare fatal case of anaphylactic shock occurring after intramuscular injection of diclofenac is presented here.

A 51-year-old man with a history of severe back pain was prescribed NSAIDs; diclofenac, in particular, had been prescribed two years before his terminal presentation. Acute onset of dyspnea followed by cyanosis of the lips and respiratory failure were described immediately after his most recent intramuscular injection of diclofenac. Unsuccessful rescue efforts included oral administration of betamethasone, intramuscular injection of adrenaline, and cardiopulmonary resuscitation. A complete postmortem examination was performed two days after death. No putrefaction phenomena were evident. External examination was unremarkable. Subpleural petechiae and heavy lungs with white foam in the main bronchi were observed on internal examination. The laryngotracheobronchial tree was unremarkable except for a mild edema of the glottis. The heart appeared normal, with conical shape and epicardial fat mildly represented. Coronary arteries were normal; luminal occlusions were excluded. All tissue specimens were formalin-fixed, paraffin-embedded, and stained with hematoxylin and eosin. Acute polyvisceral stasis, mild cerebral edema, and myocardial interstitial edema were noted. Splenic and adrenal intraparenchymal hemorrhage was observed. Acute pulmonary edema mixed with areas of acute pulmonary emphysema was evident. To estimate the mast-cell population, immunohistochemistry using anti-tryptase antibody as a mast-cell specific marker on 5mm-thick paraffin sections was performed. Pulmonary mast cells were identified and quantified, and a great number of degranulating mast cells with tryptase-positive material outside were observed. Toxicologic analysis on blood and urine therapeutic and non-therapeutic drugs was performed using gas chromatography/mass spectrometry with negative results. Serum levels of mast cell b-tryptase from femoral blood showed serum values of 43.6mg/l (normal value  $\leq 10$ mg/l). The cause of death was certified as cardiorespiratory failure due to anaphylactic shock induced by intramuscular injection of diclofenac.

In conclusion, this is a rare case of fatal anaphylactic shock due to intramuscular injection of diclofenac. This presentation is intended to bring awareness that although diclofenac is a safe and widely used drug, severe and potentially fatal anaphylactic reactions may occur with its use.

#### **Diclofenac, Anaphylactic Shock, Tryptase**



## H114 Tri-Allelic Patterns of Short Tandem Repeats (STRs) on D21S11 and Penta D Observed in Three Paternity Testing Cases With Down Syndrome Diagnosed Children

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**Learning Overview:** The goal of this presentation is to show that the use of STRs for DNA profiling can assist in the diagnosis of numerical aberrations of some chromosomes, such as 21 and 18, and as it is considered fast and sensitive, it could be used for prenatal identification of chromosomal trisomy in a short time in comparison with conventional cytogenetic methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that DNA profiling with STR amplification is recommended for a Down syndrome diagnosis, in addition to its main use in paternity and forensic cases.

DNA profiling is a routinely utilized method for determining the paternity and maternity of alleged parents to a child of concern. Many paternity cases are referred to the Paternity and Kinship Division of the Medico-Legal Directorate (MLD) in Baghdad in order to resolve paternity issues and/or to establish identity cards. Blood samples are typically taken from each family member and direct Polymerase Chain Reaction (PCR) is used for DNA amplification; PCR products are then run on the genetic analyzer, and the data is analyzed with GeneMapper® ID Analysis software. The genetic analyses in paternity testing and in forensic casework is based on the detection of STRs, which are small sequences of DNA made up of repeating units of 2–6 nucleotides.<sup>1</sup> Normally, two alleles appear at each locus (i.e., one from the father and the other from the mother; one peak for homozygous genotype and two peaks for heterozygous genotype). Chromosome number aberrations can sometimes be detected during DNA typing. The current study reports three separate cases that were referred to MLD in 2018 for paternity testing; in each case, three alleles appeared at two loci (i.e., D21S11 and Penta D, which is also located on chromosome 21) of the DNA profile for the tested children. The trisomy of chromosome 21 (all or a critical portion) is the most commonly detected form of numerical chromosome abnormality and is the cause of Down syndrome, in which significant intellectual disability and developmental delay with distinctive phenotypic features are seen in affected children. The incidence of Down syndrome is approximately 1/800 newborns.<sup>2</sup>

In 95% of chromosome 21 trisomy cases, the extra chromosome 21 has a maternal origin, while the paternal origin is responsible for the remaining 5%. These cases mainly result from non-disjunction during meiosis II.<sup>3-5</sup> The maternal origin of trisomy 21 is found to be correlated with aging (i.e., >35 years of age).<sup>6,7</sup> Trisomy 21 is known as the most common chromosomal abnormality for the live-born as well as the most prevalent cause of developmental intellectual disability.<sup>5</sup> The use of STRs for DNA profiling has many advantages in forensic and paternity cases.<sup>1</sup> Additionally, the current study shows they can assist in the diagnosis of numerical aberrations of some chromosomes like 21 and 18. Utilizing STRs for DNA profiling is fast, sensitive, and could be used for prenatal identification of chromosomal trisomy in a relatively short time in comparison with conventional cytogenetic methods.<sup>8,9</sup> Cases in which child switching after birth in the hospital is alleged are among the most frequent cases referred to MLD when the child is diagnosed with Down syndrome—yet in the majority of the cases, DNA testing reveals that those children belong to the tested parents. In conclusion, amplification of STRs could be used as a diagnostic technique for trisomy chromosome 21, along with its more standard usage in paternity and forensic cases.

*This study gratefully acknowledges the expert technical support of DNA laboratory in Paternity and Kinship Division/Medico-Legal Directorate, Baghdad/Iraq.*

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### STR, Trisomy, D21S11



### H115 A Rare Autopsy Case of Inferior Mesenteric Artery Laceration Associated With Blunt Abdominal Trauma in a Physically Abused Child

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**Learning Overview:** The goal of this presentation is for medical examiners to consider mesenteric artery laceration in addition to liver damage and intestinal rupture in cases of blunt abdominal trauma in physically abused children.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting that diverse injuries may develop as a result of blunt abdominal trauma in physically abused children.

There have been few reports of pediatric death caused by blunt abdominal trauma associated with physical abuse—and of those reported cases, the details are often unclear. This presentation will review an autopsy case of hemorrhagic shock due to inferior mesenteric artery lacerations associated with blunt abdominal trauma in a physically abused child. Moreover, the findings of the current presented case will be compared to other reported autopsy cases of blunt abdominal trauma in children.

The patient was a 3-year-old boy with a height and weight of 93cm and 14.3kg, respectively. Subcutaneous bleeding, abrasions, and small scars were observed throughout the body. There was no obvious damage observed on the skin surface in the middle abdomen; however, there was minor bleeding in subcutaneous adipose tissue, and a 6cm × 5cm × 0.5cm focus of hemorrhage within the rectus abdominis muscle was noted. In the abdominal cavity, 450ml of coagulated blood was collected. There were lacerations in the transverse mesocolon and a crush injury measuring 8cm × 9cm × 0.5cm in the mesentery of the small intestine. The inferior mesenteric artery was completely ruptured at 0.5cm from the aortic root. The transverse colon was necrotic, and there was bleeding into the mucosal membranes. Histopathologic examination revealed necrosis, neutrophilic infiltration, and hemorrhage. All organs were hypoglycemic, and the cause of death was certified as hemorrhagic shock due to laceration of the inferior mesenteric artery. Because there was no visible damage on the surface of the middle abdominal skin, strong and/or blunt abdominal trauma by a heel or similar object was suspected as the mode of injury. Based on the visual observations and histopathologic findings in the transverse colon, the subject was determined to have received the injury 3-6 hours prior to the autopsy.

In children sustaining abdominal blunt trauma, injury to organs and bowels is the main concern; moreover, vascular injury should be considered during autopsy. However, in the case of death of physically abused children, visible injury may not be found on the abdominal surface; thus, several modalities, including imaging and biochemical evaluations, should be performed during autopsy to elucidate the cause of death and pathology.

#### **Blunt Abdominal Trauma, Child Abuse, Vascular Injury**

## H116 Neonatal Pulmonary Arterial Hypertension (PAH): A Fatal Case of Noonan Syndrome (NS)

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**Learning Overview:** After attending this presentation, attendees will better understand the effects of NS on the respiratory system in association with PAH, which is an uncommon histological feature.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting that molecular genetic testing should be performed when newborns develop early severe PAH, an aggressive and often fatal condition.

NS is an autosomal dominant disorder characterized by congenital heart defects, facial dysmorphism, and intellectual disability caused by mutations in genes encoded by proteins of the RAS/MAPK pathway that regulate cellular cycle, differentiation, migration, and apoptosis.<sup>1</sup> This malformation syndrome has an estimated prevalence of 1:1,000–2,500.<sup>2</sup> The main feature of NS is a large spectrum of cardiac defects, including Pulmonary Valve Stenosis (PVS) and Hypertrophic Cardiomyopathy (HCM); the prevalence of cardiac defects in NS is about 80%–90% of cases.<sup>3</sup> Cardiac disease tends to be more progressive in infants with NS, and the earlier the presentation, the more severe the phenotype and the worse the long-term prognosis. Among those patients, there is substantial early mortality, with most deaths occurring during infancy.<sup>4</sup>

The prevalence of pulmonary pathologies in RASopathies is not as well-established as the cardiac and neurocognitive impairments. The development of pulmonary hypertension is generally a gradual and secondary effect of heart malformations.<sup>5</sup> Reported here is a case of uncommon association between NS and early-onset neonatal PAH due to a specific molecular alteration that primarily affects the arterial vessels of the lung.

A 2,150g female newborn was delivered at 34 weeks gestation to a 32 year-old primigravida. Cranio-facial anomalies were found at physical examination. Prenatal routine ultrasound detected polydramnios and biventricular hypertrophy. Family history was negative for congenital anomalies and consanguinity, and exposure *in utero* to teratogens was excluded. Over the next two months, the newborn developed significant hypoxia resulting from recurrent episodes of pulmonary atelectasia with gradual impairment of cardiac function. Echocardiogram and Computed Tomography (CT) scan showed severe HCM with evidence of PAH. Despite intensive neonatal care, the newborn succumbed to complications of acute respiratory failure at four months of age.

The external examination revealed a weight of 4,065g, length of 64cm, and head circumference of 36cm. Multiple dysmorphic anomalies were identified, including dolichocephaly with a square forehead, flat nasal bridge, low-set and posteriorly angulated ears, short neck, and nuchal edema. The internal examination showed severe right and left ventricular hypertrophy of the heart and enlargement of the pulmonary artery and its branches. The histologic examination of the lungs showed pathological changes of pulmonary arterial vessels, including medial hypertrophy, intimal proliferation with plexogenic arteriopathy, and angiomas. These findings were consistent with PAH. A comprehensive panel of genetic testing on blood samples revealed that the infant was heterozygous for the c770C>T mutation in RAF1, which is frequently associated with NS.

Several studies have reported that the RAF1 mutation produces a constitutive activation of the RAS/MAPK pathway with abnormal artery smooth muscle cell proliferation. While patients with this specific mutation are very likely to develop HCM (~85%), it also contributes to the development of an early onset of PAH.<sup>3</sup>

This presentation will provide a better knowledge on the pathogenesis of PAH in NS. An early onset of PAH, especially in newborns, is consistent with a primary genetic alteration of the pulmonary vessels rather than a progressive effect of heart malformation. Comprehensive prenatal screening for RAS/MAPK pathway mutations could allow for possible therapies targeted at the involved pathway, potentially capable of reversing HCM and delaying PAH development.<sup>6</sup>

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### Noonan Syndrome, Pulmonary Arterial Hypertension, Genetic Testing

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## H117 Sudden Unexpected Death in Childhood: A Case Report of Fatal Acute Pancreatitis

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**Learning Overview:** After attending this presentation, attendees will understand the importance of investigating a necrotizing pancreatitis in cases of sudden unexpected deaths in children.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding new knowledge about a pathology with little literature data regarding its occurrence in those of pediatric age, thus showing the importance of autopsy and histologic findings in order to understand the features of this rarely reported cause of death in children.

Necrotizing pancreatitis is a complication of acute pancreatitis wherein portions of the pancreas die and may get infected. The primary symptom of necrotizing pancreatitis is severe upper abdominal pain that radiates to the back in 50% of cases. Other related symptoms include swollen abdomen, fever, nausea, vomiting, dehydration, low blood pressure, and rapid heart rate. If left untreated, necrotizing pancreatitis may lead to bacterial infections and sepsis. Necrotizing pancreatitis is diagnosed by symptoms, but the following blood investigations are also helpful: pancreatic enzyme levels, sodium, potassium or glucose, and triglycerides levels. Abdominal ultrasound, Computed Tomography (CT) scan, and magnetic resonance imaging are additionally used to evaluate the pancreas. If these exams show that there is necrosis of the pancreas, a biopsy is necessary to test for infection. Treatments for pancreatitis include intravenous fluids, pain-relieving medication, rest, medication to prevent nausea and vomiting, and nasogastric feeding. According to a 2014 study, the best time to perform surgery is three or four weeks after the onset of the condition. However, if a person is in critical condition, surgery is mandatory to remove the necrotic or infected tissue. Early treatment is the best way to reduce the risk of necrotizing pancreatitis or other complications.<sup>1</sup>

Pediatric pancreatitis has received attention during the past few years. Numerous reports have identified an increasing trend in the diagnosis of acute pancreatitis in children and key differences in disease presentation and management between infants and older children.<sup>2</sup> Acute necrotizing pancreatitis has a variable etiology in children. CT scan is useful for the diagnosis and assessment of its severity.<sup>3</sup> Acute necrotizing pancreatitis accounts for 10% of acute pancreatitis cases and is associated with a higher mortality and morbidity. Necrosis within the first four weeks of disease onset is defined as an Acute Necrotic Collection (ANC), while Walled-Off Pancreatic Necrosis (WOPN) develops after four weeks from the disease onset. The infection of a necrotic pancreas is the most important risk factor contributing to death in severe acute pancreatitis, and it is generally accepted that infected pancreatic necrosis should be managed surgically. An infected or symptomatic WOPN requires drainage. The management of pancreatic necrosis has shifted away from open necrosectomy, as it is associated with a high morbidity, to less invasive techniques.<sup>4</sup> In contrast, the management of sterile pancreatic necrosis accompanied by organ failure is controversial. Recent clinical experience has provided evidence that conservative management of sterile pancreatic necrosis, including early antibiotic administration, seems promising.<sup>5</sup>

In order to contribute to the knowledge of the topic, the case of a 2-year-old toddler brought to the local hospital for severe abdominal pain is presented. There was no significant history reported. Severe abdominal pain, fever, nausea, vomiting, low blood pressure, and rapid heart rate were found at physical examination. Laboratory blood testing showed increased liver enzymes and amylase. The patient suddenly died after 30 minutes, before the medical staff could perform the necessary instrumental investigations.

An autopsy was conducted. The external examination showed broad marbling, especially in the lower limbs. At internal examination, a serosanguinous peritoneal effusion and a 35ml pericardial effusion were detected. Histologic findings included lungs with marked vascular congestion and limited atelectasis, reactive pulmonary hilar lymph nodes, adrenal glands with reduced medullary thickness, kidneys with focal tubular necrosis, pancreas with massive hemorrhagic necrosis, and spleen with reactive foci. The pathologist certified the case as a sudden and unexpected death caused by multi-organ failure due to septic shock in pediatric necrotizing pancreatitis.

In conclusion, this case points out that a careful histologic investigation must always be carried out in order to execute a comprehensive autopsy study, especially in the case of a sudden and unexpected death in childhood caused by a pathology that is scarcely reported in the scientific literature.

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### Sudden Unexpected Death, Childhood, Necrotizing Pancreatitis

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## H118 Multifocal Intracranial Hemorrhage in Congenital Neurosyphilis: Autopsy Findings and Literature Review

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**Learning Overview:** The goal of this presentation is to review the common clinicopathologic and autopsy findings of congenital neurosyphilis and discuss an uncommon presentation of the disease.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of congenital neurosyphilis and its manifestations.

**Background:** Congenital syphilis occurs by transplacental transmission of the spirochete *Treponema pallidum* from an infected pregnant woman to her fetus. Infection results in a spectrum of clinical manifestations, ranging from asymptomatic infection to sudden death.<sup>1</sup> The central nervous system is rarely affected in infants of treated mothers; however, if untreated, ongoing dissemination of the organism can lead to neurosyphilis.

**Autopsy:** A male infant was born at 29 weeks gestational age via cesarean section for fetal bradycardia. The pregnancy was complicated by intrauterine growth restriction and maternal history of syphilis and alcohol abuse. There were minimal signs of life at delivery, and despite resuscitative efforts, the infant passed away within an hour of birth.

Autopsy revealed a developing and normally formed infant male. The face and chest were involved by a tan-purple maculopapular rash. Small pleural and peritoneal effusions and minimal thymic hemorrhage were present, consistent with resuscitative efforts. An isolated ostium secundum atrial septal defect was identified. Bacterial and viral cultures of the lungs and cerebrospinal fluid were negative, and cultures of the blood showed mixed growth consistent with postmortem overgrowth.

Neuropathologic examination demonstrated a large subdural hematoma overlying the cerebral convexities. Scattered subarachnoid hemorrhage was present, involving the isocortex, brainstem, and spinal cord. Lymphoplasmacytic and histiocytic predominant inflammation involved the meninges. The subcortical white matter and spinal cord demonstrated severe white matter necrosis, edema, and petechial hemorrhage. The lateral ventricles were enlarged. Immunohistochemical staining for spirochetes within the parenchyma of the frontal lobe and cingulate gyrus was positive.

The cause and manner of death were certified as congenital neurosyphilis, natural.

**Discussion:** Congenital neurosyphilis generally manifests as syphilitic meningitis, with early (newborn) and late (1–2 years old) manifestations of the disease.<sup>2</sup> Despite being an acute bacterial infection, syphilitic meningitis is characterized by a mononuclear inflammatory infiltration of the leptomeninges, comprised of plasma cells, lymphocytes, and macrophages. Extension of the cellular infiltrates into brain parenchyma through Virchow-Robin (perivascular spaces), or direct extension into superficial cortex, has been suggested for those with intraparenchymal involvement.<sup>3</sup>

Syphilitic meningitis often progresses to chronic meningovascular syphilis (syphilitic vasculitis) in untreated cases. Inflammation and reparative changes cause cranial nerve palsies and hydrocephalus; over time, the vasculitis may lead to thrombosis, ischemia, and infarction.<sup>4,5</sup> Aneurysm formation and rupture with cerebral hemorrhage can occur; however, this has not been described in premature infants.

Multifocal intracranial hemorrhage represents a novel manifestation of congenital neurosyphilis, an already uncommon disease. The intracranial hemorrhage in this case has numerous potential syphilitic-mediated etiologies.<sup>6</sup> Intracranial hemorrhage has been described in infants with non-syphilitic acute bacterial meningitis and central nervous system vasculitides. Moreover, spontaneous intracranial hemorrhage is a known complication of Disseminated Intravascular Coagulation (DIC), which has rarely been associated with congenital syphilis.<sup>7</sup> Syphilis should be considered in the differential for disease processes that cause meningitis with associated hemorrhage.

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### Congenital Neurosyphilis, *Treponema Pallidum*, Pediatric Autopsy

### H119 A Peculiar Autopsy Case of Infrarenal Aortic Hypoplasia (AH) and Premature Atherosclerosis in a 13-Year-Old Male

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**Learning Overview:** After attending this presentation, attendees will be aware of the case of a young male subject affected by infrarenal AH and premature atherosclerosis with no symptoms of hypertension or lower extremities hypoperfusion, who died from a cardiac tamponade consequent to acute type A aortic dissection with intrapericardial rupture.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of this peculiar and rare pathologic condition.

AH is defined as a narrowing of the aortic lumen with consequent hemodynamic obstruction of the blood flow. It is different from coarctation, where the luminal narrowing involves a short tract of the aorta and primary involves the upper descending aorta adjacent to the site of attachment of the ductus arteriosus. While coarctation of the abdominal aorta is extremely rare, AH is an even a rarer condition and has been described both in the thoracic and abdominal aorta.<sup>1-4</sup> The precise etiology of AH is unknown; however, both congenital and acquired (inflammatory and infectious) causes have been proposed.<sup>5</sup> The clinical manifestations are related to the localization of the hypoplastic segment because, when proximal to the renal arteries, it is responsible for the activation of the renin-angiotensin system.<sup>2,6</sup> The infrarenal hypoplasia can result in hypoperfusion of the lower extremities, causing symptoms such as claudication and lower levels of blood pressure relative to the upper limbs.<sup>5</sup>

This presentation reports the illustrative case of a 13-year-old child who died soon after a sudden illness at school. The child's previous medical history was notable for only mild aortic valvular failure along with an ectatic aortic bulb. At autopsy, the main findings were: (1) type A aortic dissection with intrapericardial rupture and hemopericardium (450ml of blood in the pericardial cavity); (2) hypoplastic infrarenal aorta (1.5cm in circumference extending for more than a length of 3cm) with a calcific atherosclerotic plaque deposition; (3) cardiomegaly (heart weight: 480g) with hypertrophy of the left ventricle (left ventricular thickness: 1.8cm; interventricular septum thickness: 2cm); (4) atherosclerosis of the descending thoracic and infrarenal abdominal aorta; (5) pulmonary congestion, and (6) hepatosplenomegaly. Histopathologic examination showed separation of the tunica media layers of the ascending aortic tract with acute hemorrhagic extension and fragmentation of the elastic fibers, as well as atherosclerosis of the infrarenal aortic tract. It also revealed myocardial hypertrophy, hepatic steatosis, and thickening of the renal arteriolar walls.

In conclusion, the described case shows the association between AH of the infrarenal tract and premature atherosclerosis, consistent with the published literature.<sup>5,7</sup> The peculiarity of the case lies in the extreme rarity of this pathological condition in a male subject of such a young age, without antecedent signs or symptoms. Only in the immediate peri-mortem period was there a crushing onset of clinical manifestations, including dyspnea and cold sweating with cardiac arrest shortly thereafter; this was suspected to be due to cardiac tamponade consequent to acute type A aortic dissection with intrapericardial rupture. Ultimately, the dissection (as well as the left ventricular hypertrophy) was the result of prolonged hypertensive stimulation of the cardiovascular system, despite the localization of the aortic hypoplasia to the infrarenal aorta.

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#### Infrarenal Aortic Hypoplasia, Premature Atherosclerosis, Aortic Dissection



## H120 Histopathologic Changes in Placental Tissue Following Misoprostol Administration

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**Learning Overview:** After attending this presentation, attendees will be able to identify the histopathologic placental findings associated with misoprostol administration and utilize the information for the investigation of cases with clandestine misoprostol use.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the methods necessary for recognizing and diagnosing cases of clandestine misoprostol administration.

**Background:** Misoprostol is a prostaglandin analog commonly used alone or in conjunction with other medications to induce abortion.<sup>1</sup> Although misoprostol is only intended for medical abortion in the clinical setting, there is now greater access to the drug through online websites.<sup>2,3</sup> Women may visit these websites and purchase the drug without a prescription, allowing for self-abortions to be performed at home without the supervision of a medical doctor. In one survey, 1.2% of roughly 9,500 patients reported home misoprostol use to terminate a pregnancy, although this number was likely underestimated due to confusion over what misoprostol is and reluctance to report what is considered a crime in some states.<sup>3</sup> In the forensic setting, this fear of committing a crime may likewise contribute to a history of misoprostol use being withheld, leading to death certification as intrauterine fetal demise of unknown etiology in the absence of significant gross or histologic findings, as is often the case. Currently, the literature on the identification of clandestine misoprostol use is limited; however, there is some evidence suggesting tablet preparations of misoprostol when administered vaginally may leave refractile deposits within the placenta.<sup>4</sup>

**Purpose:** The aim of this study is to correlate microscopic placental findings in cases with known misoprostol administration.

**Methods:** A retrospective review of placental cases submitted for surgical pathologic examination over the period of a year was performed. Clinical data was collected through the electronic medical record, including maternal age, gestational history, current pregnancy data (e.g., complications, trimester), and misoprostol administration (i.e., whether administered, dose/formulation, and route of administration). The cohort was then divided into cases with no misoprostol administration, low-dose misoprostol administration, and high-dose misoprostol administration. Surgical pathology slides were reviewed for histopathologic changes, including presence or absence of refractile material, qualitative volume of refractile material present on a scale of 0 (none) to 5 (greater than 50% of tissue with deposits), histopathologic qualities (cotton fibers, amorphous material, etc.), and location of deposits (maternal or fetal surfaces, membranes, or placental disc).

**Results:** Cases with vaginal misoprostol administration were more likely to have refractile, polarizable amorphous material present. The refractile material was commonly associated with purple, dense coral-shaped deposits. The refractile material and deposits when identified were predominantly in the placental membranes and on the maternal surfaces. Refractile material consisting of cotton fibers and other debris were present in nearly all cases at varying volumes.

**Conclusions:** Microscopic examination of the placenta in cases with misoprostol administration consistently show the presence of refractile polarizable amorphous material and/or purple, dense coral-shaped deposits. The deposits described are similar in appearance to that reported for microcrystalline cellulose and crospovidone, both of which represent material used in the manufacture of tablets.<sup>4,5</sup> No standardized toxicological testing procedure is available in the United States for the identification of misoprostol due to its rapid half-life; future study of this data set will include microanalysis of unstained slides containing the refractile deposits with the goal of specific identification of the material and developing a method for confirming the presence of misoprostol in placental tissues.

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### Misoprostol, Placental Histology, Abortion



### **H121 Peripartum Cardiomyopathy (PPCM): A Forensic Approach**

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**WITHDRAWN**





### H122 Investigating DNA Methylation Analysis for the Individualization of Monozygotic Twins

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**WITHDRAWN**



### H123 Pediatric Dorsal Root Ganglia Hemorrhages in a Resuscitated Canine Mauling

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**Learning Overview:** The goal of this presentation is to highlight the importance of fundoscopic and complete spinal cord examinations in young children.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reinforcing the necessity for routine postmortem ocular and spinal cord examinations of children in traumatic and non-traumatic deaths.

Interpreting autopsy findings in pediatric deaths can be challenging due to the complicated, evolving, and occasionally conflicting body of literature regarding the specificity of certain findings. Reported here is a case of a fatal, witnessed, canine mauling that highlights the importance of a thorough autopsy to include fundal, brain, and spinal cord examination with inclusion of spinal nerve roots and dorsal root ganglia.

A 6-month-old African American male infant with a past medical history of prematurity was placed and buckled by five-point harness car seat on the living room floor by his babysitter, who then briefly left to unload her vehicle. The infant was subsequently mauled by an adult boxer mix and suffered numerous lacerated puncture wounds of the face and scalp. The mauling was witnessed in its entirety by a relative of the babysitter. He was transported by private vehicle to a local medical center and lost pulses shortly after arrival. He was intubated and return of spontaneous circulation was achieved after approximately 45 to 60 minutes of resuscitative efforts. He was flown to a level one trauma center with loss of pulses en route. He presented in cardiopulmonary arrest and was subsequently pronounced after approximately 45 additional minutes of resuscitative efforts.

External autopsy findings included a combination of lacerated puncture wounds of the face and head consistent with canine bites. Indirect ophthalmologic examination showed retinal hemorrhages of the left eye. There was fracture of the nasal bone and left orbital plate, as well as associated osseous punctures of the left temporal and parietal skull with an obvious concave deformity. Internally, there was patchy subdural hemorrhage, multifocal subarachnoid hemorrhage over the bilateral superior temporoparietal lobes, and puncture wounds of the left frontal and temporal lobes. A complete spinal cord resection with attached dorsal root ganglia by posterior approach revealed multifocal dorsal root ganglia and nerve root hemorrhages of all regions, most pronounced in the lumbar spinal cord, and confirmed in histologic sections.

Some authors previously ascribed specificity to retinal hemorrhages and dorsal root ganglia/nerve root hemorrhages of the cervical spinal cord as it relates to possible abuse head trauma (shaken baby syndrome) with hyperextension/hyperflexion injury of the neck.

With thorough scene investigation and interviews, the witness stated that there was no shaking in the attack, and that the attack simply consisted of frantic biting of the head only with the infant relatively stationary in the five-point restraint car seat. This study proposes that dorsal root ganglia hemorrhages in young children are a non-specific finding and may be seen as a component of hypoxic/ischemic reperfusion injury. This case highlights the importance of routine complete spinal cord examination and fundal examinations in young children and also highlights the importance of being cautious of ascribing diagnostic specificity to retinal hemorrhages or dorsal root ganglia/nerve root hemorrhages.

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#### Mauling, Spinal Cord, Infant



### H124 The Postmortem Microbiome: An Evaluation of 16S Ribosomal RNA (rRNA) Profiles

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**Learning Overview:** After attending this presentation, attendees will learn how to use hypervariable regions V1 and V2 of the 16S rRNA gene to determine the postmortem interval.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the determination of time of death using amplicon Length Heterogeneity-Polymerase Chain Reaction (LH-PCR) and capillary electrophoresis.

The ability to quantify the microbial diversity within a human postmortem microbiome is fundamental to the elucidation of molecular functions after a human dies. Before the advent of advanced forensic molecular techniques, the study of the functions of microbial communities has been challenging to investigate due to their different scales (e.g., spatial and temporal). However, with increased use of high-throughput DNA sequencing, combined with bioinformatic analyses, these limitations are quickly diminishing. To date, the “gold standard” of DNA analytical approaches involve a multifaceted process of metagenomic sequencing that is moderately costly and time-consuming to perform. One effective method used to screen microbiomes is to generate a community DNA profile using fluorescent-based DNA profiling methods such as amplicon LH-PCR followed by capillary electrophoresis. Due to the natural occurrence of insertions and deletions within 16S rRNA genes, different length amplicons are generated when the DNA is amplified using universal primers. The hypervariable regions, V1 and V2, demonstrate an extensive range of amplicon lengths that represent the minimum microbial diversity. While any one amplicon could represent different nucleotides, thus different microbial species, it enables forensic scientists to efficiently survey the dynamics of a community under differing postmortem growth conditions. This approach is beneficial because it allows for the rapid production of a genetic pattern, or snapshot, of the bacteria present at the time of sampling, which is an important aspect to consider when choosing samples for downstream metagenomic sequencing.

In the present, first-of-its-kind postmortem microbiome study, 32 samples (brain, heart, liver, and spleen) from eight cadavers of various causes of death from criminal cases were investigated. The results reveal two noticeable trends that affect the number of postmortem microbes and conceivably (1) the frequency of proliferation, (2) the time since death, and (3) the cause of death. Therefore, time-since-death rates of proliferation may prove advantageous for forensic science in determining postmortem interval. LH-PCR is a valid, rapid technique that can monitor these dynamics and, therefore, screen postmortem samples that would be of interest for sequencing analyses. Furthermore, this method is a very inexpensive technique to screen samples to compare community patterns over time and between individuals.

#### 16S rRNA, Postmortem Microbiome, Capillary Electrophoresis



### H125 Simplified DNA Barcoding Strategy for Forensically Relevant Blow and Flesh Flies

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**Learning Overview:** The goal of this presentation is to propose a simplified DNA barcoding strategy for identifying insects commonly encountered in casework at the Harris County Institute of Forensic Sciences (HCIFS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a simplified method for DNA barcoding as a tool for medicolegal death investigations.

Accurate insect identification is critical to their use in the estimation of Time Of Colonization (TOC) and Postmortem Interval (PMI) during medicolegal death investigations. Insect specimens are currently identified by evaluating morphologic characteristics as indications of particular taxonomic groups; however, this process is limited because immature life stages typically lack distinguishing morphologies. Identification may be achieved by rearing live specimens; however, this process is time-consuming, labor-intensive, and not always successful.

These deficiencies may be addressed through molecular identification by DNA “barcoding,” wherein DNA sequences from unknown samples are matched to references. This technology enables identification of immature specimens, may be performed without specialized forensic entomology training, and requires equipment common to forensic genetics laboratories. DNA barcoding has been demonstrated in numerous entomological surveys of forensically relevant species; however, the technology has not been implemented for medicolegal death investigations. This is due in part to deficiencies in the technology: no single primer set is capable of distinguishing all of the diverse species important to forensic investigations. Instead, multiple primer sets and sequencing reactions are utilized to maximize the species that may be identified.

Proposed here is a simplified DNA barcoding strategy for identifying insects commonly encountered in casework at the HCIFS. The strategy comprises sequencing and phylogenetic analysis of a single barcoding fragment amplified from the mitochondrial COI locus. Using verified reference specimens, this study shows that the DNA barcoding strategy enables statistically supported identification of species previously encountered in the HCIFS medicolegal death investigations, in particular, members of blow fly genera *Lucilia*, *Calliphora*, *Chrysomya*, *Phormia*, and *Cochliomyia*, the flesh fly genus *Blaesoxipha*, and the scuttle fly genus *Megaselia*, and is effective for immature insect specimens, for example, pupal exuvia, larva, and pupa. Identification is demonstrated for immature specimens collected during past HCIFS medicolegal death investigations for which species-level identification was undetermined by morphology. Future work will include elucidating inter-specific and intra-specific sequence variations of local blow and flesh fly populations to provide further statistical support for identifications and validating the DNA barcoding assay for casework application.

#### DNA Barcoding, Postmortem Interval, Forensic Entomology



### H126 The Effect of Mass-Generated Heat on Larval Development: Implications for Postmortem Interval (PMI) Estimates

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**Learning Overview:** After attending this presentation, attendees will better understand the impact of mass size and heat generation on larval development rates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the need for mass temperatures to be incorporated into minimum PMI (mPMI) estimates using larval development.

Observed to arrive at a body within minutes of death, blow flies (Diptera: *Calliphoridae*) are often used by investigators as a tool to aid in the determination of time since death, also known as PMI. Estimating the mPMI based on larval age involves identifying the blow fly species, reconstructing the thermal history at a scene, and modeling the rate of development. Previous research has shown that a strong positive relationship exists between mass size and heat generation, with aggregations composed of as few as 1,200 larvae producing temperatures that are significantly warmer than ambient.<sup>1</sup> Since blow fly larvae are known to experience faster rates of development at warmer temperatures, one would expect the size of the feeding mass to influence the rate of development. However, there are ongoing difficulties with incorporating this concept into mPMI estimates, with very few studies quantifying the relationship between mass size and development. The goal of this research was to determine whether larvae feeding in different-sized masses develop at different rates. The research also identifies which stages of development are most affected by mass size and to what extent.

Aggregations ( $N=21$ ) containing either 300, 1,200, or 2,500 *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae) larvae were reared in a laboratory on racks of lamb ribs at a constant ambient temperature of 22°C ( $\pm 1^\circ\text{C}$ ). A Forward Looking Infrared Radar (FLIR) T425 thermal imaging camera was used to record mass temperatures every five minutes throughout the feeding stages of development. Larvae were sampled every hour and instars were determined by examining the posterior spiracles, which allowed developmental rates to be monitored. At two points during development, 70 hours and the start of the dispersal phase, larvae had their lengths and fresh weights recorded. Data were analyzed using the statistical package R (version 2.12.1).

Results showed a strong positive relationship between mass size and the amount of heat generated by the aggregation ( $p \leq 0.001$ ), with temperatures rising as masses increased in size. While the duration of the egg and 1<sup>st</sup> larval instar phases remained constant regardless of mass size, larvae did show an accelerated rate of development during the 2<sup>nd</sup> and 3<sup>rd</sup> instar when sampled from increasingly larger masses. Accelerated development coincided with the achievement of peak temperatures in the masses. These faster growth rates resulted in the larger aggregations entering the post-feeding phase of development an average of 13 hours earlier than smaller, cooler masses. Physical measurements taken from larvae sampled at 70 hours development showed that individuals feeding in larger masses were significantly longer and heavier than those sampled from smaller aggregations ( $p \leq 0.001$ ), with larvae from the aggregations of 2,500 individuals having a mean length and weight of 13.90mm and 38.81mg, respectively, compared to 12.34mm and 25.99mg in the 300 masses. This provided further evidence of faster growth rates. However, by the time larvae reached the dispersal phase, these differences in length and weight no longer existed ( $p=0.088$ ).

This research highlights the need to incorporate mass temperatures into forensic casework when using larval development to estimate the time of death. Larvae sampled from large masses, particularly during the 3<sup>rd</sup> instar phase, could appear older (re: size) than they actually are due to the accelerated rates of development experienced under warmer conditions. If this is not taken into account, it could result in an overestimation of the mPMI. Future research should focus on identifying how other variables influence heat generation, as well as finding ways to estimate the size of a mass and, hence, its thermal history at a scene.

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#### Maggot Masses, Blow Fly Development, Postmortem Interval



### H127 The Impact of Postmortem Microbiota on *Lucilia Sericata* Development

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**Learning Overview:** After attending this presentation, attendees will have a better appreciation of the impact of selected postmortem bacteria on *Lucilia sericata* development.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the influence of postmortem microbiota on necrophageous insects, which can be of use when evaluating the minimum postmortem interval.

When a putrefied corpse is discovered, several issues arise for the investigators, including providing an estimation of the postmortem interval. Different methods are used to determine an answer to this question, such as the study of the necrophageous insects when they are present. Even though the bacterial communities are abundant on a decomposing body, interactions between insects and the postmortem microbiota are still little known and underestimated. The purpose of this study is to clarify the influence of bacteria that are present during decomposition on the development of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), a forensically relevant blow fly species.

Several *L. sericata* larvae were sampled on decomposing human corpses and their Excretion/Secretion (ES) fluids were extracted. The bacteria present in the ES fluids were identified using cultural methods and mass spectrometry. Among them, two strains were chosen for confrontation during larval development: *Proteus mirabilis* and *Providencia alcalifaciens*. *L. sericata* eggs were sterilized using successive baths of sodium hypochlorite and 70% ethanol solution. After hatching at 25°C, the larvae were transferred on artificial diets in three different conditions: two groups mono-inoculated with either *P. mirabilis* or *P. alcalifaciens* as well as a control. The diet (Columbia yeast agar) was selected to be as favorable for insects as for bacterial proliferation. Larval development was observed from first instar to puparial stage, which was allowed using sterilized sand around the plates. All insects were followed at 25°C until adult emergence. More than 750 larvae were tracked for every studied condition (the experiments are still in progress). For each replicate of every tested condition, ten larvae were sampled every eight hours during five consecutive days between first instar and post-feeding stage. Larval development and survival were compared between each bacteria and the control. The preliminary results are in favor of differences concerning the early phases of the larval growth, but more replicates are still needed.

During decomposition, bacteria such as *P. mirabilis* and *P. alcalifaciens* (both naturally present in the human intestinal microbiota) are proliferating and interacting with the other present organisms. This study will allow a better understanding of the biotic and abiotic factors involved with decomposing human remains.

### Taphonomy, Postmortem Microbiota, Forensic Entomology



## H128 A Survey of Bacterial Communities in Soil Associated With Porcine Remains

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**Learning Overview:** After attending this presentation, attendees will better understand how porcine remains affect the soil microbiome. This information will assist forensic scientists in improving the estimation of the Postmortem Interval (PMI) based on microbial evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the bacterial communities associated with carrion-deposited soil samples and their importance in forensics. This information will help attendees gain a better understanding of carrion resource utilization and improve the precision of PMI estimation using entomological and bacterial evidences.

Estimating the PMI is one of the crucial parts of a death investigation. Immediately after death, algor, rigor, and livor mortis are often used as a PMI estimation method. However, these methods often lose their reliability after 72 hours postmortem. In recent years, soil has become a useful tool in determining this timeline. As bodies are often buried underground or deposited on land, soil may serve as a logical means of estimating time since death. Soil acts as a sink or filter for water, gases, and other resources in the environment, potentially making it a new identifying factor in estimating the PMI. As decomposition progresses to the bloat stage, it releases nutrients and microbes into the underlying soil, altering the soil microbial community composition.<sup>1</sup> Understanding how the microbiome of soil is altered as time progresses will aid the forensic science community in enhancing PMI estimation methods.

This study characterized bacteria associated with carrion-deposited soil samples by using 16S recombinant DNA (rDNA) MiSeq® sequencing. Six porcine cadavers were left to decompose naturally on top of pristine soil. Soil samples were collected directly under the body and three meters away from the body. This repeated each day for a week, then once a week after that for a total of eight weeks. DNA was extracted from the soil samples using the DNeasy® PowerSoil Kit and was amplified and sequenced for Variable region Four (V4) of 16S rDNA using the dual-index MiSeq® sequencing strategy as described by Kozich et al.<sup>2</sup> Sequences were then analyzed using mothur version 1.39.4, and statistical analysis was performed using R version 3.4.0.<sup>3,4</sup>

In general, the total DNA quant value obtained from soil under porcine remains was higher than the DNA quant value obtained from soil at the control sites. After all quality control steps, a total of 4,518,957 sequence reads (average read per sample=55,820) was generated from all samples. Preliminary results show that Acidobacteria and Actinobacteria are among the most prominent phyla observed in soil associated with porcine remains.

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### Soil Microbiome, Necrobiome, 16S rDNA

## H129 Cranial Hyperostosis and Neuropsychiatric Disorders: Is There a Correlation? A Comparison Between Forensic Cases and a Review of Literature

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**Learning Overview:** After attending this presentation, attendees will understand the role of frontal hyperostosis in the genesis of neuropsychiatric disorders.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the need to investigate the role of hyperostosis as a potential association and predictive factor of neuropsychiatric diseases.

Cranial hyperostosis is an idiopathic condition characterized by an increase in skull thickness. Frequently, it involves the frontal bone and the anterior fossa. Hyperostosis, and particularly Hyperostosis Frontalis Interna (HFI), has been described in association with psychosis, depression, Parkinsonism, executive function disorders with memory loss, and inappropriate behavior. Only case reports are published in forensic literature. A correlation has been hypothesized with the so-called Morgagni-Stewart-Morel (MSM) syndrome, characterized by HFI and metabolic and neuropsychiatric disorders.<sup>1</sup> However, to date, there is no general agreement on the existence of the MSM syndrome as the pathogenesis of the phenomenon remains unclear.

The aim of this study is to examine the possible correlation between cranial hyperostosis and neuropsychiatric disorders, comparing the published literature to the findings in three forensic autopsy cases. In each case, the morpho-volumetric analysis of bone structure and brain was performed at autopsy (i.e., measuring thickness of the skull, diameters of the cavities, presence of bone spurs, weight and size of the brain, thickness of the cortex, and brain analysis by Virchow cutting). Toxicologic analysis was performed. An investigation was carried out regarding previous hospitalizations and psychiatric disorders, incorporating information from family doctors and psychiatrists. Finally, a literature search was performed utilizing the PubMed<sup>®</sup> National Center for Biotechnology Information (NCBI) search engine and the key words “cranial hyperostosis” and “psychiatry” OR “psychiatric disorders.” Obtained literature was reviewed.

**Case 1:** A woman suffering from diabetes and schizoaffective disorder (depressive type) suffered an exacerbation of delirium, hallucinations, and dysphoria over the past few months. She required assistance, support, and continuous reassurance from physicians; she was not reliable and was unable to function effectively independently. She died following a suicidal fall from a height. At autopsy, HFI was noted with the presence of multiple, diffuse bone spurs, located in the anterior cranial fossa, which protruded toward the brain. The absence of fractures was due to greater resistance of the bone structure of the skull. The brain weighed 1,090 grams with softening areas and punctiform hemorrhagic areas on the thalami and the cerebellum.

**Case 2:** A woman suffering from severe dementia and diabetes was admitted with a pertrochanteric fracture of the left femur after an accidental fall. Reportedly, her dementia had diminished her cognitive abilities significantly enough that it compromised her ability to perform daily activities. She died due to massive pulmonary embolism following the femur fracture. At autopsy, frontal cranial hyperostosis was noted. The brain showed a flattening of the cerebral convolutions and a reduction in the thickness of the cortex (1,040 grams).

**Case 3:** A man was found dead due to a suicidal gunshot injury. At autopsy, Diffuse Cranial Hyperostosis (HCD) was evident with an overall reduction in brain volume (approximately 1,000 grams). There was a large subarachnoid and intracerebral hemorrhage due to the firearm. His clinical history was significant for depression with suicidal ideation.

These cases highlight disorders arising in the context of hyperostosis, including prefrontal syndrome, depression, and neurodegenerative diseases (dementia). These disorders coincide with those described in the literature, except for suicidal behavior.<sup>2,3</sup> Literature review showed that the potential role of frontal hyperostosis in the genesis of neuropsychiatric disorders so variable in their etiology, pathophysiology, and symptoms is still controversial. The most accepted hypothesis is that an abnormal release of hormones (e.g., estrogens) has a decisive role in the genesis of a “neurometabolic” syndrome. The overproduction of estrogens results in the growth of frontal bone (a known hormonal target), neuropsychiatric disorders, and metabolic diseases with greater prevalence in women.<sup>1</sup> In the current study, two of the three reported cases involved women suffering from diabetes. Additionally, the growth of sharp bone spurs on the endo-cranium could also exert a “disturbing” role on the frontal and prefrontal lobes, contributing to the prefrontal syndrome associated with the hyperostosis. In the context of neurodegenerative disorders, literature review also showed a correlation between HFI and a decrease in brain volume.<sup>4</sup> The current study illustrates the need for a statistical large-scale collection of anatomic data in psychiatric patients. Such a survey could prove critical in establishing structural anatomical data as a potential predictor of psychiatric disorders, such as prefrontal syndrome, dementia, and depression with suicidal ideation.

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### Forensic Sciences, Cranial Hyperostosis, Psychiatry

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## H130 Patient Misinterpretation of Findings Leads to Suicide

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**Learning Overview:** The goal of this presentation is to emphasize the importance of performing an autopsy in the wake of an unconfirmed medical diagnosis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance of post-suicide autopsy as it pertains to patient-physician communication and its role in a tragic suicide.

Approximately 14 in every 100,000 people commit suicide every year.<sup>1</sup> In 2008, 22.6% of suicides were attributable to physical health problems.<sup>2</sup> This represents a particularly relevant avenue of intervention for health care professionals. Physicians are privy to patients' most intimate health information and thus should be aware of a possible mental health crisis. When handling such matters in particular, physicians' words carry great weight. Occasionally, physicians can become numb to the sway they have on the lives of patients. Patient misinterpretation of their doctors' words can have devastating consequences. This concept is crystalized in the case of a patient who, due to a misunderstanding of information provided, committed suicide because she presumed she had been told she had cancer.

A 75-year-old woman with a history of chronic obstructive pulmonary disease, atrial fibrillation, emphysema, and hypertension presented to an Emergency Department with complaints of worsening constipation, epigastric pain, and nausea of three-days' duration. Physical exam was non-specific. A computed tomography scan revealed bilateral cystic and solid adnexal masses concerning for ovarian neoplasms. She was referred to an oncologist. Two days later, before meeting with the oncologist, her husband tried to wake her—only to find her dead in her bed, with a suicide note present. It read: "SORRY DEAR NO MONEY FOR CHEMO WON'T DO ANY GOOD I LOVE YOU! GET WELL." Subsequent medicolegal autopsy revealed a relatively healthy elderly woman. The cause of death was the toxic effects of multiple drugs, including propafenone. The manner of death was suicide. The ovarian tumors were benign cystadenofibromas, characterized by benign fibrous stroma and cysts lined by serous-type or mucinous epithelium, with no evidence of borderline or malignant cells.

In order to prevent similar tragic deaths, more patient-to-provider information checkpoints should be implemented. This would entail having patients repeat back their understanding of their diagnoses. Patients can also overestimate their understanding of care information. An interactive communication loop helps mitigate these gaps.

One technique for this is Ask Me 3, which identifies three main questions the patient should be able to answer at the end of a healthcare visit: (1) What is my main problem?; (2) What do I need to do?; and (3) Why is it important for me to do this? Use of the Ask Me 3 technique incorporates important attributes of adult learning—multiple modalities, reinforcement, and personalization—and has been linked to better health outcomes.<sup>3</sup>

In addition to communication concerns, the presented case demonstrates the importance of performing an autopsy when a patient has received a recent unconfirmed medical diagnosis, particularly if that diagnosis somehow played a role in death. A breakdown in physician-patient communication was uncovered as a result of a postmortem examination. Knowledge of the autopsy findings will alert the physician to a possible need for communication adjustment.

Some might argue that an autopsy in the case of a suicide is unnecessary in the presence of an obvious mechanism of mortality. This is true to an extent, but, in general, it does not apply to suicides by overdose. According to the Forensic Autopsy Standards promulgated by the National Association of Medical Examiners, autopsies should be performed on cases of suspected drug overdose.<sup>4</sup>

Forensic pathologists should consider performing autopsies in cases of sudden death occurring in situations with ambiguous medical diagnoses, particularly if such diagnoses may have precipitated a suicide. In addition to either confirming or refuting the decedent's supposed underlying pathology, performing autopsies in such cases may assist in revealing any possible lapses in communication between health care providers and patients.

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### Communication, Suicide, Autopsy



### H131 Colonization Rates of Barnacles (Crustacea: Cirripedia) on Different Fabrics as a Tool for Forensic Investigation of Human Remains in a Marine Environment

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**Learning Overview:** After attending the presentation, attendees will understand how long barnacles require to colonize four different fabrics (cotton, velvet, satin, and neoprene) in marine waters off the coast of Perth, Western Australia. The outcome of this research identifies which fabric was the most desirable substrate for colonization.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information pertaining to the colonization, settlement preference, and growth of barnacles on various types of clothing in a marine environment. This research will contribute toward increasing the accuracy of the minimum Postmortem Submergence Interval (minPMSI) of clothed human remains in a marine environment.

**Background/Introduction:** The estimation of the time since death (i.e., minimum Postmortem Interval [minPMI]) is an important aspect of a forensic investigation in a terrestrial environment and is even more challenging when a body is found submerged or floating in a marine environment. Human remains discovered on land involve an estimation of the minPMI based on the presence of carrion insects, generally blow flies (Diptera: Calliphoridae). In the marine environment, the time spent underwater by the body (minPMSI or Floating Interval [FI]) is required for an accurate minPMI. The presence of barnacles (Crustacea: Cirripedia) are one parameter that have come under recent scrutiny, due to their colonization and permanent settlement on human remains and accompanying items, such as shoes and clothing. Research on the useful nature of barnacles in forensic investigations is scarce and, to date, has not considered the different clothing materials that may be associated with human remains.

**Objectives and Methods:** The current research is focused on the colonization, settlement preference, and growth of barnacles on cotton, velvet, satin, and neoprene. Polystyrene Floats (PF), covered with four types of fabric, and uncovered Polystyrene Float Controls (PFC) were submerged over a period of six months. The investigation was located off the coast of Perth, Western Australia. The aims of this research were: (1) the identification of marine species colonizing the fabrics, with special attention paid to barnacles; (2) the identification of which fabric provides the most desirable environment for colonization; and (3) the understanding of which fabric affects the growth rate of the different species of barnacles.

**Results and Discussion:** Results revealed the presence of three species of barnacles, in varying numbers and sizes. *Balanus trigonus* Darwin was found to be the most abundant and frequently present. The two other species, *Amphibalanus reticulatus* (Utinomi) and *Amphibalanus variegatus* (Darwin), were less abundant. The colonization process of the barnacles happened rapidly, with colonization observed in the first month (28 days) on neoprene and PFC. Statistical analyses were used to determine significant relationships between barnacle diameter (measure of growth) and fabric. Overall, the most favorable substrates that were colonized by barnacles were neoprene and PFC, followed by satin. Cotton showed a low colonization rate, possibly due to the partial deterioration of the material over time, and velvet showed an inconsistent colonization rate. The settlement preference of barnacles on all fabrics and the PFC was on the bottom half of the floats (away from sunlight exposure) and areas where the fabric folded. This study is the first to provide data to support the estimation of PMSI of clothing fabrics that may be associated with human remains in Australian marine waters.

#### Barnacle, Colonization, Postmortem Interval



### H132 The Applicability of FLIR® Thermal Imaging of Swine Decomposition During the Louisiana Summer

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**Learning Overview:** After attending this presentation, attendees will have gained insight on the use of thermal imaging in forensic entomology and taphonomic studies. The goal of this presentation is to characterize the thermal sources and effects of external (ambient and carcass surface) and internal heat units (maggot mass heat, carcass tissues) on blow fly development and postmortem estimations. This presentation will illustrate the potential differences between observed thermal heat units and those hypothetically available at Cadaver Decomposition Islands (CDIs).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how the incorporation of FLIR® C2™ thermal imaging can provide real-time documentation of thermal units at CDIs and human remains recovery sites.

Postmortem estimations based on insect evidence are often determined by the summation of thermal units at a crime scene to predict the energy budgets required to complete specific insect life stages present on human remains. Forensic entomology studies have increasingly focused on improvements to accumulated degree day calculations and thermal summation models, with particular emphasis on: (1) biological thresholds for growth; (2) maggot mass metabolic heat; and (3) the curvilinear portion of insect development.<sup>1,2</sup> The aim of this research was to incorporate infrared thermal imaging to field studies of large vertebrate carrion, as well as to illustrate the potential usefulness of FLIR® thermal imaging to both decomposition research and law enforcement communities.

Louisiana has a humid subtropical climate with long, hot, humid summers and short, mildly warm winters.<sup>3,4</sup> Whereas, summer daily ambient temperatures typically exceed 32°C with carcass temperatures often approaching biological maximums for blow fly development when large maggot masses are present (greater than 50°C). FLIR® infrared thermal imaging can provide real-time documentation of carcass surface temperatures, as well as areas of elevated internal temperatures due to maggot mass metabolic heat. A preliminary study utilizing infrared imaging as a “non-invasive tool” for documenting maggot mass temperatures on swine carrion in Australia was conducted in 2014.<sup>5</sup> However, those authors briefly studied only one juvenile swine carcass in the field (7d) and one juvenile swine in a temperature-controlled room (9d).

This research was conducted using three fresh adult swine carcasses (~45–60kg each) placed on the ground in a hard-bottom flatwoods forest at Southeastern Louisiana University’s Outdoor Classroom in July 2019. Each sampling event included manual sampling of insects, digital photography, and multiple temperature measurements within the CDI. Temperature data included: (1) FLIR® C2™ compact professional thermal imaging system (infrared camera) to document carcass surface temperatures and maggot mass activities within the carcass; (2) dual digit temperature probe for internal carcass temperatures, maggot mass heat, soil-carcass interface, soil and ambient temperatures; and (3) climatology data from Hammond Municipal Airport weather station. Accumulated degree day estimations were calculated for multiple indicator species using both observed thermal units and regional airport climatology data. Earliest blow fly colonizers for all three swine carcasses included: *Chrysomya megacephala* (F.), *Cochliomyia macellaria* (F.), *Lucilia coeruleiviridis* Macquart, and *Phormia regina* (Meigen).

The FLIR® C2™ infrared camera provided the following for each sampling event: (1) infrared images (isotherms); (2) relative humidity isotherms; and (3) digital photographs for comparisons. All infrared images were analyzed using FLIR® Tools+ Imaging Software (2016). Nine to ten carcass and CDI regions were analyzed per sampling event: mouth (anterior), ear (dorsal head), forelimbs, abdomen, hind limbs, posterior, beneath the carcass, wounds, and adjacent ground/leaf litter (control). Thermal recordings from digital temperature probes were analyzed against the FLIR® output and isotherms were compared for humidity levels. The addition of FLIR® Tools+ output contributed valuable information to the overall understanding of the microenvironment of CDIs and the role of temperature and humidity throughout vertebrate decomposition.

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#### *Cochliomyia Macellaria*, Thermal Imaging, Forensic Entomology



### H133 Maternal Death by Fire and Fetal Carboxyhemoglobin Levels

*Nicole D. Lee, MS\*, Western Michigan University, Kalamazoo, MI 49007; Theodore T. Brown, MD, Kalamazoo, MI 49008*

**Learning Overview:** After attending this presentation, attendees will understand: (1) the physiology of acute carbon monoxide exposure and carboxyhemoglobin levels, (2) the existing research from animal models and limited case studies on maternal carboxyhemoglobin levels and concurrent fetal levels, (3) how paired maternal and fetal carboxyhemoglobin levels vary based on the type of carbon monoxide exposure, and (4) how the findings from the present case fit into prior findings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a better understanding of the significance of carboxyhemoglobin levels in mothers and fetuses in the setting of both acute and chronic carbon monoxide exposure.

The effect of maternal carbon monoxide exposure on fetuses is an underreported topic. The relationship between maternal carboxyhemoglobin levels and fetal carboxyhemoglobin levels has yet to be fully explained, especially in cases of acute, non-smoking-related exposures. Data from animal model experiments on rabbits and ewes demonstrated a lag in rising fetal carboxyhemoglobin levels after acutely exposing the mother to carbon monoxide. Eventually, over several hours, the fetal levels exceed the mother's and reached equilibrium approximately 10% higher. A search of previously published studies on humans only revealed seven paired reports of maternal and fetal carboxyhemoglobin. Presented here is a pregnant female involved in a residential fire where carboxyhemoglobin levels were tested in both the mother and fetus.

An 8-month pregnant, 35-year-old woman died in a house fire caused by an overloaded extension cord. Postmortem examination documented extensive fire-related changes, which included singed hair, diffuse charring of the skin, skin splits, and soot deposition of the airways. The skin splits were predominantly on the forehead, left temple, perioral region, chin, upper chest, bilateral upper extremities, and lower abdomen. There was dense soot in the nares, oral cavity, and trachea, and scant soot in the bilateral mainstem bronchi and proximal esophagus. The muscles, body cavities, and organs had a bright red-pink discoloration. The decedent's carboxyhemoglobin level was elevated at 51.3%. Her cause of death was inhalation of products of combustion and thermal injuries. The fetus had neither trauma nor malformations. Fetal heart carboxyhemoglobin level was 4.9%.

The research on what fetal carboxyhemoglobin levels occur at certain maternal carboxyhemoglobin levels is unclear. While there are some studies on chronic maternal exposure to carbon monoxide (mostly through smoking) and paired fetal and maternal carboxyhemoglobin levels, there are very few case studies available regarding acute carbon monoxide toxicity and concurrent maternal and fetal carboxyhemoglobin levels. The levels found in this case provide insight into this relationship by corroborating previous findings that demonstrated a lag between acute and severe maternal exposure and a corresponding rise in fetal levels.

#### Carboxyhemoglobin, Pregnant, Fire



### H134 The Mouse Thanatomicrobiome Is Highly Variable in Early Postmortem Intervals

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**Learning Overview:** The goal of this presentation is to present data regarding the mouse thanatomicrobiome and discuss its application as a potential alternative to current postmortem interval determination. This presentation will also provide evidence the mouse model system may be applicable to human thanatomicrobiome studies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a potential alternative to current postmortem interval determination. This is especially relevant as determining an accurate postmortem interval is a key component of many crime scene investigations.

The postmortem microbiome of internal organs is referred to as the thanatomicrobiome, and microbial changes that occur within may have potential forensic applications. As compared to microbes on the surface of a decomposing body (epinecrotic microbiome), microbial signatures associated with internal organs are less susceptible to environmental changes and may represent a valid means of determining a postmortem interval. Although the thanatomicrobiome remains poorly understood, recent advances in the field of metagenomics have provided the necessary technology to better investigate the microbiome of this complex environment.

This laboratory previously investigated the mouse liver thanatomicrobiome to determine if it would be a suitable model system comparable to a human. Using 7–21-day postmortem mice, a *Clostridium* effect was observed similar to that reported in human cadavers. Furthermore, as the postmortem interval increased, the species richness of the liver also increased, suggesting that decomposition is mediated by a wide variety of microorganisms. However, questions remained regarding the makeup of microbial signatures in earlier postmortem intervals. To fill this gap of knowledge, the liver thanatomicrobiome of pre-seven-day postmortem mice was examined.

Mice were sacrificed using CO<sub>2</sub> asphyxiation and immediately placed in covered containers maintaining proper air flow to allow the natural decaying process to take place. Three separate time intervals were analyzed: one, three, and five days postmortem, in addition to control groups (zero and seven day postmortem). Livers were dissected and stored at -80°C until DNA was extracted with a Zymo Research Tissue/Insect Miniprep kit following the manufacturer guidelines. Subsequently, the V3 hypervariable region of the 16S ribosomal RNA (rRNA) gene was amplified and sequenced on an Illumina MiSeq® platform. Bioinformatic analysis of the resulting data identified predominant organisms present down to the level of genus.

Day one postmortem mice consistently revealed *Escherichia* and *Bacteroides* predominated. *Escherichia* levels were extremely variable (61-1%) while *Bacteroides* levels were much more consistent (3-4%). Although day three mice consistently demonstrated that genera in the family *Clostridiaceae* increased along with *Bacteroides* as its predominant members, this trend did not continue as the day five postmortem mice demonstrated high levels of *Escherichia*. At day seven postmortem, the *Clostridium* effect was widely seen as earlier determined. The observed variability seen in days one through five extended into the Shannon Species Diversity Index, which measures species richness. Values obtained were extremely variable, and no discrete trends were observed as compared to the 7-21 day postmortem mice. Taken together, these data suggest the liver thanatomicrobiome is highly variable in the initial days postmortem and individual host differences may significantly affect the rate of decomposition.

#### Thanatomicrobiome, Postmortem Interval, *Clostridium* Effect



### H135 A Fatal Hemorrhage From a Periumbilical Wound: A Case of Stabbing or a Rare Bleeding From a Caput Medusae?

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**Learning Overview:** After attending this presentation, attendees will have learned about a rare complication of portal hypertension in a context of Hepatitis C Virus (HCV) -related liver cirrhosis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing attention to the importance of conducting a complete autopsy examination, supported by histological studies, in all patients with portal hypertension in order to identify skin wounds that could be a sign of an ectopic varix rather than a case of stabbing. This presentation will also raise awareness in the clinical community for understanding a complication of portal hypertension which, without a proper treatment, could be fatal.

Portal hypertension is a serious complication of chronic liver disease. Varices are the most common clinical manifestations, and variceal bleeding is the predominant cause of mortality in patients with portal hypertension. The most common site of varices is the lower esophagus, but they may occur at any location where there are portosystemic anastomoses, including the periumbilical venous plexus. Periumbilical varices are known as “caput medusae.” Deaths due to exsanguination from periumbilical variceal rupture are rare with only two such fatalities reported in the literature.

A 55-year-old man was found dead in a hotel room. He was lying on the floor near the bed in a prone position. The body was surrounded by a large pool of blood that extended (in drips and stripes) to the bathroom floor. The pattern of blood suggested that the man had moved from the bathroom to the bedroom, where he then died. The external examination of the body revealed a small round wound close to the umbilicus. On pressing the abdomen, blood was seen to flow from the wound, thus establishing the origin of the hemorrhage. No further injuries were detected. A review of the decedent’s medical history revealed that he was affected by type II diabetes mellitus, HCV-related liver cirrhosis, and portal hypertension. He also had a liver tumor, which had been treated with chemotherapy, and was waiting for a transplant.

Autopsy revealed a cirrhotic liver with firm parenchyma. Evidence of portal hypertension included dilatation of the vena cava, splenomegaly, and intact esophageal varices. No blood was recovered from either peritoneal cavity, nor was evident in the gastrointestinal tract. A relevant finding was the presence of abnormally dilated and convoluted veins in the subcutaneous tissue of the umbilical region (i.e., caput medusa). The abdominal wound depth was established with the help of a probe, showing that the injury extended deep into the subcutaneous fat and the periumbilical veins. All these autopsy investigative findings served to exclude a stabbing injury. Histologic sections showed that the abdominal wound consisted of epidermidis located on top of an ectatic venous vessel with a focally interrupted wall. In the subcutaneous tissue, there was a widespread presence of ectatic vessels of medium to large caliber. These histologic findings confirmed that one of the periumbilical varices had ruptured and connected with the overlying skin through a fistula that was likely formed due to inflammation. Ultimately, the cause of the death was attributed to massive hemorrhage due to periumbilical varix rupture with cutaneous fistula formation in a patient with portal hypertension attributable to HCV-related liver cirrhosis.

In conclusion, only by careful and complete autopsy—including histologic analysis, medical record review, and circumstantial scene investigation—is the correct final diagnosis obtained in extremely rare and complicated cases such as this. Moreover, this case highlights the importance of evaluating for the presence of possible varices in less-common locations when dealing with patients affected by portal hypertension.

#### Caput Medusae, Portal Hypertension, Fatal Hemorrhage



### H136 Brain Damage and MicroRNA (miRNA) Dysregulation: An Experimental Study

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**Learning Overview:** After attending this presentation, attendees will have a better comprehension of miRNAs as a useful tool, both in the clinical setting and in medicolegal investigation. Particularly, this experimental study focused on brain damage, analyzing the miRNAs expression values in four selected groups of cadavers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing evidence about the expression levels variation of the specific miRNAs (miR-21, miR-34, miR-124, miR-132, and miR-200b) involved in the control of important target genes that regulate the neuronal apoptosis and neuronal stress-induced adaptation.

Brain damage and/or dysfunction as a sequelae of multiple different conditions is considered an important field of research for the scientific community. Recent studies have focused on side effects related to aging of the Central Nervous System (CNS). Moreover, a growing number of investigations have been conducted, analyzing the CNS effects of stroke and drug use/abuse. The identification of specific circulating and/or tissue biomarkers that could indicate brain injury remains challenging, however. Since 2007, miRNA technology has become an integral part of research; the scientific community has frequently investigated utilizing miRNA as potential molecular biomarkers for several diseases. Today, miRNA dosage has become an essential tool in several clinical applications.

This experimental study focused on miRNA expression in cases of brain injury. MiRNA is a well-known diagnostic tool, both in the clinical setting and in medicolegal investigations. Previous studies have demonstrated that specific miRNAs (i.e. miR-21, miR-34, miR-124, miR-132, and miR-200b) control important target genes involved in neuronal apoptosis and neuronal stress-induced adaptation. Thus, in this experimental setting, their expression was evaluated in four selected groups of decedents in which five males were selected for each group (i.e., Anabolic Androgenic Steroid [AAS]; propionate testosterone) abuser, drug abusers (cocaine), ischemic-stroke-related deaths, and aging damage in elder decedents who died from other neurological causes).

Total RNA, including miRNAs, was isolated from Formaldehyde-Fixed Paraffin-Embedded (FFPE) samples using the RecoverAll™ Total Nucleic Acid Isolation Kit with minor modifications. For miRNA profiling, the TaqMan Advanced miRNA Assay was used. Complementary DNA (cDNA) was obtained following TaqMan® Advanced miRNA Assays User Guide (Publication number 100027897 Rev. C). Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was performed using the StepOnePlus™ Real-Time PCR System, and raw data were analyzed using the relative software (version 2.3). Expression fold changes were computed using the  $2^{-\Delta\Delta Ct}$  calculation.

Study results demonstrated that drug abuser and AAS abuser groups showed a higher expression of miR-132 and miR-34, suggesting a specific pathway in consumption-induced neurodegeneration. Conversely, miR-200b and miR-21 dysregulation was linked to age-related cognitive impairment. Finally, ischemic stroke-related deaths were associated with an alteration in miR-200b, miR-21, and miR-124; significantly higher levels of this last expression were strongly sensitive for ischemic damage. Moreover, these results suggest that these expression patterns could be studied in other biological samples (e.g., plasma, urine) in subjects with brain injury linked to aging, AAS abuse, drug abuse, and stroke to identify reliable biomarkers that could be applied in clinical practice. Further studies with larger samples are needed to confirm these interesting findings.

### Anabolic Androgenic Steroids (AASs), Brain Injury, MiRNA Dysregulation



### H137 Under Pressure: A Small Italian Town Under Attack

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**Learning Overview:** The goal of this presentation is to report the case of a young girl killed in front of her school by the explosion of a rudimentary bomb. The application of a multidisciplinary forensic approach showed the death was due to a primary blast lung injury.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reporting an example of an analytic approach to guide the forensic pathologist in the diagnosis of primary blast injury in explosion deaths, which are increasing events in civilian populations.

Blast injuries are physical traumas due to explosions. They are a frequent event in war zones, but may increasingly be seen in civil settings due to terrorist attacks. Primary blast injury is defined as being attributable to the blast wave effects. Air-filled organs, such as lungs, the gastrointestinal tract, and middle ears, are most frequently affected because the blast wave easily compresses them. The diagnosis of primary blast injury can be challenging for the forensic pathologist as the absence of external injuries may lower the suspicion for investigating internal lesions.

This presentation reviews the death of a 15 year-old girl secondary to the explosion of a handmade bomb. The event occurred at around 7.45 a.m. in front of a high school in an Apulian town in Italy,. The bomb was hidden behind a garbage can placed near the school gates and was operated remotely. A group of nine students were wounded; among them, a 15 year-old girl who died shortly after arrival at the hospital. An autopsy was performed. However, before autopsy the forensic pathologist requested a total body Computed Tomography (CT) scan, which documented the presence of multiple pulmonary lacerations and contusions of the right lung, bilateral pleural effusion, and a massive left pneumothorax with hypexpansion of the left lung. External examination showed multiple excoriations spread over the body and particles of a blackish color fixed in the facial epidermis. Internal examination confirmed the CT findings and revealed the presence of fractures of the posterior arches of the eighth and ninth left ribs and a fracture of the left scapula. Histologic investigations performed on the lungs with hematoxylin-eosin staining showed pleural lacerations, subpleural hemorrhages, acute pulmonary emphysema, acute alveolar septal ruptures, and massive endoalveolar hemorrhages. The immunohistochemical examination of skin samples, performed using anti-Heat Shock Protein (HSP) antibodies (i.e., HSP 27, HSP 70, HSP 90), confirmed the presence of heat alteration of the skin. Skin samples stained with Perls' histochemical method (to demonstrate the presence of iron) and with the sodium (Na) -rhodizonate solution (to demonstrate the presence of lead, barium and antimony), were observed in polarized light and in phase contrast microscopy, using a quantitative analysis of images. The reactions were positive.

The absence of significant external lesions, in conjunction with the pulmonary histologic alterations and immunohistochemical evidence, led to the conclusion that death was due to acute respiratory injury secondary to the architectural subversion of the pulmonary parenchyma as a direct consequence of the detonation of an explosive bomb (i.e., primary blast lung injury). The perpetrator responsible for the massacre claimed to have triggered the bomb in anger due to a scam he suffered. The prosecutor's office identified the event as a terrorist act; the perpetrator was sentenced to life imprisonment. No other cases like this one have been reported in Italy.

In cases of explosions, decedents should undergo radiologic evaluation prior to the autopsy examination. Such examination allows for the identification of several findings that may not otherwise be evident during the autopsy (e.g., pneumothorax) and can guide the forensic pathologist in focusing on organs of interest. In suspected primary blast lung injury, histologic studies are mandatory to confirm typical evidence (e.g., diffuse alveolar overdistension and interstitial hemorrhage). Immunohistochemical methods may provide additional information about the biochemical changes that can occur after the heat damage, which are in other ways complicated to analyze; additionally, this method can provide information about the chemical elements contained in the explosive mixture. Overall, the diagnosis of blast injury-related deaths can be challenging because of their low frequency in the civil population and non-specific presentation that can mislead forensic pathologists inexperienced in this type of case. This presentation aims to propose a model of an analytical approach to these types of deaths.

### Blast Injury, Explosions, Analytical Approach



### H138 A Cold Bone Heart: A Rare Case of Death Due to Acute Myocarditis in a Subject Suffering From Chronic Constrictive Pericarditis

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**Learning Overview:** The goal of this presentation is provide a suitable methodological approach to cardiac-related death due to suspected myocarditis occurring in subjects suffering from chronic constrictive pericarditis instrumentally ascertained. This study focuses on macroscopic and histological findings used to reach the diagnosis of the cause of death, with particular regard to the immunohistochemical assessment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the methodological approach employed in rare cases of fatal acute myocarditis in subjects with diagnosed chronic constrictive pericarditis and the importance of conducting an exhaustive immunohistochemical study with CD45, CD3, CD4, CD8, CD20, CD15, and CD68 antibodies in order to obtain a reliable postmortem diagnosis of myocarditis.

Myocarditis may present with a wide range of symptoms, ranging from mild dyspnea or chest pain, resolving without specific therapy, to cardiogenic shock and death. The onset of acute myocarditis in a substrate of patients with pre-existing chronic constrictive pericarditis is a sporadic occurrence.

A 48-year-old man with a history of chronic constrictive pericarditis of unknown etiology presented to his family doctor with respiratory difficulties, asthenia, and fever with shivering. The doctor prescribed antibiotic therapy, recommending a second evaluation after a few days. However, the day after, the patient was taken to the hospital due to a sudden drop in blood pressure. Electrocardiogram showed diffuse ST elevation, while laboratory tests showed increased levels of cardiac necrosis markers. Echocardiogram revealed the presence of a hyperechoic calcific pericardial formation that circumferentially enveloped the heart, as well as ventricular hypertrophy with a deficit in lateral kinetics and an ejection fraction of 30%. Cardiac ventriculography confirmed the circumferential pericardial calcifications, while coronary angiography revealed no arterial stenoses. Notwithstanding the diagnostic efforts of the physicians, the patient died the following day of unclear causes. Following a complaint of medical malpractice to the judicial authority, a complete autopsy was performed.

At autopsy, gross examination of the heart revealed it to be encased by a fibrocalcific rind, thus confirming the clinical history of chronic constrictive pericarditis. Moreover, the heart had increased dimensions with dilated ventricular chambers and increased parietal pericardial thickness. The coronary arteries were essentially unremarkable without stenosis. Examination of remaining organs was unremarkable. Hematoxylin-Eosin (H&E) -stained histologic sections highlighted a polyvisceral stasis, as well as the presence (in the cardiac samples) of marked fibrotic thickening of the pericardial leaflets with areas of infiltration of white cells. These white cells were also present in the myocardium, disrupting the myocardial architecture. Multiple foci of Contraction Band Necrosis (CBN) involving the sub-endocardial layers were found. To better characterize the myocardial cellular infiltrates, an immunohistochemical evaluation was performed using antibodies against leukocytes (CD45), the main leukocyte subpopulations (i.e., CD3, CD4, CD8, CD20, CD15), and macrophages (CD68). The cellular infiltrate demonstrated strong positivity for CD45 (+++), moderate positivity for CD68 (++), mild positivity for CD8 (+), and negativity for the remaining markers.

Given these results, according to the Dallas criteria, the patient's death was attributed to massive acute myocarditis arising in the setting of chronic constrictive pericarditis. Given the rapidity of the clinical course, the prosecutor determined no malpractice on the part of the physicians; indeed, they had performed multiple diagnostic tests and provided the correct supportive therapy as indicated by the most authoritative literature on the subject.

#### Acute Myocarditis, Chronic Constrictive Pericarditis, Immunohistochemistry



## H139 Using Culturomics to Investigate the Mouse Thanatobiome

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**Learning Overview:** The goal of this presentation is to demonstrate how culturomics can complement the metagenomic investigation of the mouse thanatobiome.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how the isolation and identification of living microbes from the thanatobiome will increase understanding of its culturable bacterial diversity. This knowledge will complement existing molecular data to aid in determining if the thanatobiome can provide an alternative to existing methodologies for Postmortem Interval (PMI) estimation.

Forensic microbiology is a rapidly expanding field that has many broad applications in forensic science. Recent advances in DNA sequencing and computing technologies (e.g., metagenomics) have provided researchers with a molecular toolkit to explore a multitude of diverse forensic questions—for example, investigating microbial changes that occur postmortem using next generation DNA sequencing to yield a profile of the thanatobiome, or microbes found associated with internal organs after death. Despite these technological advances and data obtained, many questions remain regarding how to interpret the results and their direct forensic application.

While the use of metagenomics has revealed a complex and diverse thanatobiome that changes with Postmortem Interval (PMI), all interpretations are based on a small piece of DNA and not the organism itself. Indeed, the recent revival of culture-based microbiology, referred to as culturomics, is gaining momentum in the scientific community. Being able to study a living microbe rather than information from a piece of DNA may provide additional opportunities to understand the relationship between the thanatobiome and PMI estimation.

Previously, this laboratory has been examining the usefulness of a mouse model system to study the thanatobiome. Using an Illumina® MiSeq® platform, DNA sequencing of the V3 hypervariable region of the 16S ribosomal RNA (rRNA) gene indicated that *Clostridium* species dominated as early as seven days postmortem. As we continue to gain a better understanding of the mouse thanatobiome and its usefulness in PMI determination, a culturomics-based approach was initiated to start obtaining information based on the direct isolation of microbes from the mouse thanatobiome.

Mice were sacrificed by CO<sub>2</sub> asphyxiation and placed in sealed containers that allowed air flow for up to seven days. Livers were harvested at PMIs of two and seven days. The harvested livers were homogenized in a saline solution and put through a series of serial dilutions. These dilutions were plated on pre-reduced blood agar plates, placed into an anaerobic chamber, and left to incubate at 37°C until bacterial colonies appeared (about three days). Organisms were isolated and total DNA purified using the ZR Fungal/Bacterial DNA MicroPrep kit. Subsequently, the 16S rRNA gene was amplified by Polymerase Chain Reaction (PCR), followed by DNA sequencing. The basic local alignment search tool (Basic Local Alignment Search Tool [BLAST]) was utilized for bacterial identifications.

The obligate anaerobe *Clostridium* dominated the isolations at both PMIs, followed by the facultative anaerobes *Escherichia* and *Shigella*, which could not be discriminated from each other. Less abundant facultative and aerotolerant anaerobes were also identified. Although a limited number of bacterial isolates were examined, this data supports the *Clostridium* effect dominating the culturable thanatobiome, although no discrete trends were seen. Further examination into the non-*Clostridium* population is needed to ascertain if culturomics can support the metagenomic investigation of the thanatobiome and its usefulness in PMI determination.

### Thanatobiome, Culturomics, Postmortem Interval



### H140 An Accidental Asphyxiation by Christmas Lights in a Patient With Schizoaffective Disorder

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**Learning Overview:** After attending this presentation, attendees will have reviewed an unusual case of accidental asphyxiation with an atypical ligature and familiarized themselves with the impact of psychiatric illness on mortality, specifically regarding accidental and suicidal deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community in terms of recognition of an unusual ligature not previously reported in asphyxial death, differentiating accidental from suicidal death in persons with psychiatric illness, and evaluating the role of psychiatric illness in a shortened life span and in accidental asphyxia.

Patients with psychiatric illnesses have been shown to have shortened life expectancy and increased rates of mortality from all causes of death. Accidental and suicidal deaths are significantly increased in psychiatric patients, especially those with schizophrenia, when compared to the background population.<sup>1</sup> An illustrative case report is presented.

A 62-year-old woman with multiple psychiatric diagnoses, including schizoaffective and substance use disorders, was found dead by her brother in midsummer in her locked trailer home. Her body was in a condition of moderately advanced decomposition, consistent with the interval observed (i.e., last seen alive five days earlier). Medical and social history included repeated admissions to local inpatient psychiatric wards; she had been the subject of numerous calls to police regarding aggressive or irrational behavior when home. Her brother was her primary caretaker. At the scene, the interior temperature was measured at 105°F. Christmas lights lined the bedroom ceiling, on three of the four walls. The decedent's body was supine on the floor near the bed, entangled in Christmas lights around the neck and torso.

At autopsy, external examination was notable for dried brown-black fluid emanating across the cheeks from each corner of the mouth. The Christmas lights were deeply embedded horizontally around the neck, crossing at the left side of the neck without a knot. One string passed around her upper arm and shoulder; the other string wrapped twice around her torso. After removal of the Christmas lights, a corresponding ligature mark was identified in the exposed dermis; internal examination identified a linear pale area on the undersurface of the platysma muscle and across the superficial strap muscles, without other evidence of injury. Identifiable natural disease included emphysema, atherosclerosis, and remote tubal ligation. Death was attributed to accidental asphyxia from a Christmas light ligature. The contribution of heat was unclear, but likely not significant in the asphyxia. As it seemed likely that she should have been able to untangle the Christmas lights if free of mental illness, schizoaffective disorder was deemed a contributing cause.

Psychiatric illnesses are recognized to place patients at a higher risk of accidental death, as well as suicide and natural death. The literature examining the mortality gap between decedents with and without psychiatric illness has given rise to a number of theories for the discrepancy, including negative side effects of antipsychotic medications, increased tobacco use, unhealthy lifestyle, delayed diagnosis and suboptimal treatment of physical illness, and increased risk of unnatural death.<sup>1</sup> Internet and medical library searches have not identified any previous case reports of intentional or accidental asphyxiation via Christmas light ligature. A case of accidental asphyxial death has been reported in an inebriated 67-year-old man without psychiatric history, who was found dead in a sitting position, with the collar of his sweater hanging off the brake handle of a motorcycle.<sup>2</sup> A schizophrenic woman in her sixties who was judged to have asphyxiated herself on an intraoral handkerchief may have been responding to internal stimuli secondary to her psychiatric illness (suicide) or may have accidentally blocked her airway while attempting to stop the bleeding from a recently avulsed canine tooth.<sup>3</sup> In either scenario, her psychiatric illness was relevant to death. This case report identifies another unusual mechanism of accidental asphyxial death in a woman with psychiatric illness.

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#### Asphyxiation, Psychiatric Illness, Christmas Lights



### H141 When the Autopsy Is the Only Possibility to Obtain a Diagnosis: The Investigation of a Rare Case of Aortopulmonary Window (APW)

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**Learning Overview:** The goal of this presentation is to report the autopsy investigation of a neonatal death due to a rare congenital defect, APW.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by outlining the role of forensic pathology in the management of a difficult task, such as the diagnosis of congenital heart defects.

APW is one of the rarest congenital heart defects, accounting for 0.5% of all congenital heart defects. The malformation is caused by incomplete development of the aortopulmonary septum during fetal life. APW can be isolated or seen as a part of a wide variety of other cardiac anomalies, such as atrial or ventricular septal defects, patent ductus arteriosus, Berry syndrome, pulmonary atresia, aberrant subclavian artery, and tetralogy of Fallot. Patients with APW have increased pulmonary flow, due to the presence of a left-to-right shunt, which leads to pulmonary hypertension in early life and is associated with a poor prognosis in the absence of surgical correction.

This is the report of a case of a male newborn delivered via caesarean section at full-term (39 weeks) who rapidly developed cyanosis, poor reactivity, and hypotonia, necessitating partial resuscitation and oxygen therapy. The quick clinical deterioration resulted in rapid transportation to an advanced hospital where a diagnosis of respiratory distress was made. Despite the intensive care, the symptoms worsened, and the newborn died about 15 hours after birth. An autopsy investigation was performed in order to establish the cause of the symptoms and death.

At autopsy, the cervical, thoracic, and abdominal organs were removed en masse, according to the Letulle technique. After external examination, the heart, lungs, and aorta were separated as a block, and subsequently fixed in formalin. Gross examination after formalin fixation showed an increased heart volume and a pseudo-aneurysmal dilatation of the pulmonary artery. Sectioning of the great vessels highlighted a wide communication between the aorta and pulmonary artery due to lack of an aortopulmonary septum; the defect was located about 3mm before the origin of the left subclavian artery. Main branches of the aortic arch were normal, except for a stenosis of the left subclavian artery approximately 6.6mm from its origin. The findings identified during the postmortem examination made it possible to identify the cause of death as acute cardiopulmonary insufficiency due to a congenital heart defect with a left-to-right shunt (i.e., APW).

Despite advancements in medical technology and practice, congenital heart diseases are still encountered at autopsy of many stillbirths, newborns, and infants. The rarity and the rapid clinical evolution of these conditions—as well as the low likelihood of antemortem diagnosis in many cases—constitutes a challenge for the forensic pathologist. The current case report emphasizes the importance of choosing the appropriate autopsy technique and the need of training in the detection of congenital heart defects, especially in situations of antemortem misdiagnosis or where ancillary methods, such as forensic radiology, are unavailable.

#### Congenital Heart Defects, Aortopulmonary Window, Autopsy Investigation



### H142 A Comprehensive Review of the Pathology of Blunt Traumatic Spinal Cord Injury (TSCI) Resulting in Early Fatality

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**Learning Overview:** After attending this presentation, attendees will understand the pathologic changes associated with blunt TSCI, as well as the connection between specific pathologic changes and underlying mechanisms of injury.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing an epidemiologic profile for blunt impact spinal cord trauma in a large urban environment and by identifying patterns of injury that may be seen in spinal cord trauma due to blunt impact.

**Introduction:** TSCI is associated with significant neurologic morbidity and mortality. Although the annual incidence of TSCI is estimated at 54 cases per million people per year, data from the National Spinal Cord Injury Statistical Center do not typically include cases with a fatal outcome at the scene of injury.<sup>1</sup> Moreover, the pathologic features of blunt TSCI occurring at the moment of impact are less well-described than secondary changes as a complication of initial tissue disruption. Therefore, characterization of the pathologic changes associated with early lethality is needed to better understand the mechanisms of TSCI.

**Methods:** This study aimed to characterize the neuropathologic profile of blunt impact TSCI resulting in a fatal outcome at the incident location. An electronic database at the City of New York Office of Chief Medical Examiner was queried for all blunt force trauma deaths undergoing full autopsy between 2003 and 2018. All cases with spinal trauma, either in isolation or in combination with other blunt injuries, were identified. Autopsy reports were reviewed by two board-certified forensic pathologists. Exclusion criteria were: (1) any known survival period after injury, (2) any history of spinal disease, and (3) TSCI due to a non-blunt mechanism (e.g., sharp force or gunshot trauma).

**Results:** Two hundred twenty cases of blunt TSCI, resulting in a fatal outcome at the incident location, were retrospectively identified over a 16-year period. The median age was 46.5 years; 77.72% were male and 22.28% were female. Manners of death were accident (80.9%), suicide (10%), homicide (3.7%), and undetermined (5.4%). The most common mechanism of injury was motor vehicle collision (44.1%), followed by fall from standing height (29.5%), and descent from height (12.3%). Ethanol was the most frequent substance on toxicologic testing (33.6%). Atlanto-occipital dislocation was seen in 15.45% of cases and showed an association with transportation-related fatalities in young individuals. Most spinal fractures were single-level injuries (73.6%). Spinal cord findings were available in 83.6% of cases and included an admixture of contusions (32.65%), lacerations (51.7%), softening (31.29%), and crush injury (8.2%). Up to 20.1% of cases had a normal spinal cord on gross examination. Of the 55.9% of cases with available data on spinal hemorrhage, epidural (59.3%) and subarachnoid (25.2%) compartments were the most frequently affected.

**Conclusion:** Presented here is data from a large retrospective series of blunt impact TSCI in association with early lethality in a large urban setting. By broadening the TSCI population to include cases from a forensic setting, a spectrum of findings associated with primary TSCI, that are not typically captured by large TSCI registries, was identified. Also identified was a possible connection between specific mechanisms of injury and the resulting pathology. These findings highlight the role of forensic pathologists in characterizing the spectrum of TSCI with implications for the development of preventative strategies.

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#### Spinal Cord, Blunt Impact, Trauma



### H143 The Histomorphology of Cranial Fracture Healing: Preliminary Observations

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**Learning Overview:** After attending this presentation, attendees will have an understanding of important histological features of cranial fracture healing and a proposed method for evaluating them in four differently stained slides.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the field's knowledge of how cranial fractures heal and the cells and tissues involved in this process. In addition, this work is laying the foundation for a method to evaluate healing cranial fractures.

Understanding how cranial fractures heal and time-since-injury are important goals of forensic pathology and anthropology, especially as these biological responses relate to the context of accidental versus non-accidental injury. The scientific literature does not provide useful data or methods for estimating times since injury of cranial fractures. As such, this project has two primary research goals: (1) documenting the histomorphology of healing cranial fractures, and (2) establishing histomorphologic phases of cranial fracture healing.

Histologic sections from subadults and adults with known cranial fractures and/or surgical interventions of varying healing times have been collected and stained with: hematoxylin and eosin, Masson's trichrome, alcian blue hematoxylin/orange G, and Russell-Movat pentachrome. These stains were chosen for their ability to highlight different elements of bone healing. Specifically, evaluators are charged with identifying the following cells and tissues associated with bone healing: hematoma, pigment-laden macrophages, fibrin, inflammation, fracture edge morphology, mesenchyme/loose connective tissue, fibroblasts and/or fibrocytes, fibrous connective tissue/collagen, new capillaries, cartilage matrix, bone matrix, bone resorption, woven bone, lamellar bone, and reversal/cement lines.

To prepare samples for histology, the following methodology is employed: a gross sample of the fracture is removed (approximately 20–30mm); the sample is decalcified using either nitric acid, Ethylenediaminetetraacetic acid (EDTA), or Hydrochloric Acid (HCL); sections of the fracture are cut and the tissue is paraffin embedded; four thin sections are cut; and each section is stained and mounted on a glass slide. The slides are digitized and uploaded to a digital microscopy database. Glass slides are placed in four-slide kits and assessed by three randomly selected evaluators. As previous investigators have found variation in bone healing between the outer and inner tables, three different zones were identified for evaluation: the outer table, diploë, and inner table. For each slide, the evaluators assess the presence, absence, and quantity of the cells or tissues listed above. A separate evaluation form is used to assess each zone and stain; as such, each evaluator collects data on 12 different evaluation forms for each sample, resulting in 396 observations on a single sample (and 1,188 total observations by three evaluators per sample). When all slides in a set are assessed, evaluation forms are entered into a database for use in various statistical analyses. Analyses include evaluation of intra- and inter-observer variability; patterns in the presence, absence, and relative quantity of cells and tissues; and correlation of various cells and tissue.

Early findings suggest that HCL is an inferior method for decalcification, compared to EDTA and nitric acid. In addition, the cell and tissue types differ in juvenile versus adult individuals. Additionally, evaluators are noting considerable variability in the type of tissues present in the fracture gap among age-cohorts. Some of these differences include the presence of woven bone and cartilage matrix in juveniles, but these tissues appear to be absent in adult cranial injury healing. As more samples are collected, researchers will be able to provide evidence-based timelines for how different types of cranial bone injuries heal among various age-cohorts. Furthermore, the results of this research will provide a framework for forensic pathologists and anthropologists to sample, document, and classify fractures, as well as provide basic science data for future research goals of creating a method for interpretation of time-since-injury.

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#### Fracture, Histology, Time-Since-Injury



### **H144 Rule Out Trauma: A Five-Year Retrospective Study on Natural Disease, External Trauma, and Manner of Death at the Harris County Institute of Forensic Sciences (HCIFS)**

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**Learning Overview:** The goal of this presentation is to evaluate the incidence rate of lethal trauma in decedents when the preponderance of investigative evidence suggests death by natural disease.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating that although a decedent may have some form of external trauma, the severity of underlying internal trauma will determine whether the manner of death is ascribed as natural or accident.

Blunt force injuries are one of the most common injuries evaluated by forensic pathologists. Many decedents will have peri-mortem blunt force injuries, and it is imperative to determine if these are the cause of death or sequelae subsequent to natural disease. Harris County, TX, is the third most populous county in the nation and the most recent HCIFS annual report (2017) showed that 4,731 medicolegal cases were received for examination. Of these, 40.4% were classified as natural deaths, 35.7% were classified as accidents, 11.1% were classified as suicides, 9.6% cases were classified as homicides, 3% were classified as undetermined, and 0.1% were classified as fetal deaths. Greater than one-third of medicolegal cases at HCIFS are natural deaths; however, initially it can be challenging to ascertain if these cases are natural deaths based on the investigative information presented or accident based on the external examination findings. Many decedents who die of natural diseases may have peri-mortem trauma after collapsing or falling. At the same time, some decedents may have a plethora of natural diseases but may die secondary to trauma. The current study investigates how many HCIFS cases with external trauma truly had internal trauma.

A five-year retrospective review of all cases autopsied between the years 2014 and 2018 was performed to identify cases wherein the decedent had external trauma, such as a head laceration or contusion, and an autopsy was performed to rule out internal trauma. Daily case logs containing pertinent investigative information on the circumstances of death were reviewed, and cases were selected based on evidence of concurrent natural disease and peri-mortem trauma, such as a head laceration, contusion, or evidence of a reported fall prior to death. Cases with clear accidental causes and severe external trauma, such as motor vehicle accidents or work accidents, were excluded. Demographic information as well as cause of death and manner of death were then analyzed.

While several of the selected cases in this study did have fatal internal injuries, such as blunt force head injuries or blunt force injuries of the torso, the majority of the decedents died from natural diseases with trauma likely as a secondary result of a sudden collapse/fall. Thus, while peri-mortem injury can lead one to contemplate about accident as a manner of death, it is crucial to correlate circumstantial data along with autopsy findings.

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#### **Blunt Force Injury, Natural Disease, Peri-Mortem Injury**



### H145 Radically Invasive Projectile (R.I.P.) Ammunition: The Projectile With No Boundaries

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of R.I.P. ammunition and its potential to create a unique pattern of injury. Attendees will also have insight into the wound tracts that the projectile fragments may take by learning the ballistics of the frangible projectile.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information regarding frangible projectiles and, more specifically, the possible extent of injury with R.I.P. ammunition. This presentation will also impact the clinical community, specifically emergency department and trauma health care providers, by improving their understanding of the potential wound tracts to guide informed medical care decisions.

The R.I.P. ammunition, a newly designed frangible bullet marketed to users of handguns as “the last round you’ll ever need,” is becoming an increasingly popular choice for self-defense rounds.<sup>1</sup> This type of ammunition, from G2 Research, is composed of a base with eight trocars that are intended to fragment upon impact, creating nine individual wound tracts with a radial dispersion.<sup>2</sup> This may create difficulties for first responders, clinicians, and forensic pathologists as the trajectory is different from that anticipated for the main projectile or base. In addition, break-up of the projectile prior to entry may result in more than one entrance wound from a single projectile. The fragmented projectile increases the number of internal injuries, resulting in the need to document and follow several wound tracks and recover multiple small retained fragments.<sup>1,3</sup> If the forensic pathologist or clinician does not have a good understanding of the ballistics of this ammunition, appropriate documentation, medical care, and projectile recovery may be missed. Few case reports have been published on R.I.P. rounds and may be overlooked by the forensic community as these publications are predominantly in the trauma and emergency medicine literature.<sup>3-5</sup>

Six cases in which the decedent had penetrating and/or perforating gunshot wounds from R.I.P. ammunition are presented. Five of the six cases were fatal, with one decedent being remotely shot by an R.I.P. ammunition round. In this latter case, the decedent died of other acute multiple gunshot wounds, thus disproving the marketing motto for this type of projectile. In all six cases, many radio-opaque bullet fragments were seen on postmortem radiographs using a LODOX® scanner. During the autopsy, several fragments were recovered from each decedent and preserved as evidence.

The R.I.P. ammunition used in these cases provides a challenge for forensic pathologists. Postmortem imaging can be extremely useful in identifying the location of each retained trocar and the base. Understanding the ballistics of the R.I.P. ammunition will assist in accurately identifying multiple wound tracts, trajectories, and projectile recovery.

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#### Frangible Projectile, Gunshot Wound, Forensic Pathology





### H146 Suicides in Cook County, Illinois: A Four-Year Retrospective Study From 2015 to 2019

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**Learning Overview:** After attending this presentation, attendees will understand the trends seen in Cook County, IL, with respect to the mechanism, seasonal variation, and demographics of suicides.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting mechanisms more prevalent in a particular demographic set and preventative measures that could be applied.

According to the Centers for Disease Control and Prevention, nearly 45,000 lives were lost to suicide in 2016 and suicide rates have increased by more than 30% in more than half of the states since 1999. Suicide is the tenth leading cause of death in the United States since 2008. Suicide mortality trends have been proven to be a beneficial public health tool. Determining high risk areas, gender prevalence, and mechanisms could assist in better allocating resources for suicide prevention. Also, this data could potentially be extrapolated and applied to other metropolitan regions as trends at the county level have been largely unexplored to date. The relationship between adverse socio-economic circumstances and suicidal behavior is well established; however, the impact of adverse collective circumstances, such as the socio-economic context where people live, is less well understood.

A retrospective search of the Cook County Medical Examiner's Office database from 2015 through April 2019 was performed for decedents in which "Suicide" was the manner of death. The case files and recorded demographic data, as well as cause of death, mechanism of death, and month of occurrence were reviewed.

**Results:** A total of 2,003 suicide cases were identified from January 2015 to April 2019 in Cook County. The top five most prevalent suicide mechanisms were hanging (673 cases), gunshot wounds (632 cases), overdoses (228 cases), jump from height (111 cases) and train-related fatalities (82 cases). A majority of the suicides occurred in the city of Chicago, as compared to the suburbs. The ages ranged from 11 to 94 years. There was a greater prevalence of suicides in males as compared to females, with 473 females and 1,530 males (1:3 ratio). The number of suicides per year was relatively stable over the course of the study period, ranging from 436 to 481 fatalities. The detailed results of the study will be presented to attendees.

**Conclusion:** Mapping county-level suicide rates provides greater granularity in describing geographic patterns of suicide and contributes to a better understanding of changes in suicide rates over time. Findings may aid in better targeting prevention efforts as well as contribute to future research on community-level risk and protective factors related to suicide mortality.

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#### Suicide Trends, Demographics, Suicide



### H147 Mississippi Organ and Tissue Recovery in High-Profile Medical Legal Cases

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**Learning Overview:** After attending this presentation, attendees will better understand organ, eye, and tissue recovery outcomes in a state with an exceptionally high homicide and infant mortality rate utilizing a mixed coroner/medical examiner system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the results of organ and tissue recovery and transplantation along with the autopsy results with respect to three medical examiner cases.

Since the development and opening of the Mississippi Organ Recovery Agency (MORA) in 1994, a common mindset among coroners regarding homicides, high-profile cases, or infant fatalities had been that donation and recovery of organs and tissues prevented accurate investigation and postmortem findings. This was due to a lack of coroner understanding regarding recovery and postmortem examination procedures. This misunderstanding continued throughout the state for nearly 20 years, which resulted in numerous families robbed of the opportunity to give the Gift of Life and/or honor the wishes of their loved one who had signed the donor registry, a legally binding, first-person authorization.

In 2017, the Office of the State Medical Examiner (OSME) of Mississippi and MORA began to develop protocols regarding the risks and benefits of organ and tissue recovery with respect to medicolegal cases. These protocols followed state statutes in addition to guidelines set by the National Association of Medical Examiners. These protocols reflected the state statutes regarding the responsibilities of the coroners throughout the state. These protocols have been successful in educating coroners and increasing their knowledge that organ and tissue recovery does not preclude an accurate medicolegal investigative process.

Over the past few years, MORA has been able to recover many medicolegal cases, ranging from high-profile homicides, unnatural infant fatalities, and unique circumstantial deaths. Recovery and donation performed by MORA has never disrupted or compromised an investigation in the state of Mississippi. This success has resulted in hundreds of families being able to turn their tragedy into a positive outcome, as well as thousands of lifesaving and healing gifts transplanted to those in need. This positive outcome is due to the support of the OSME and the collaborative protocols put into effect by all three agencies (i.e., MORA, OSME, and coroners). As a result of these protocols, the number of cases shut down by a coroner have decreased by nearly 100%. MORA and the OSME plan to continue these collaborative protocols and share the results of the successful donation outcomes combined with successful medicolegal investigations and prosecutions that were not affected by the recovery of organs, eyes, and/or tissues for the purpose of transplantation or research.

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### Transplantation, Medical Legal, Collaborative



### H148 Non-Natural Deaths on Hospital Property: A Five-Year Retrospective Review of Cases From Harris County, Texas (2014–2019)

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**Learning Overview:** After attending this presentation, attendees will understand the most common scenarios for non-natural deaths on hospital property (including outpatient clinics and doctor offices) in Harris County, TX, which includes the city of Houston and surrounding municipalities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of fatal incidents apart from adverse events of medical care that can lead to death while on hospital property. The forensic community can play a vital role in injury prevention by tracking these deaths and educating the local health care institutions about these occurrences, as well as by identifying and potentially mitigating risk factors.

The current patient safety culture in the hospital setting largely focuses on adverse events (i.e., injuries that occur due to complications of medical management rather than the disease process itself) or falls while in the hospital setting.<sup>1,2</sup> Literature delving into other non-natural deaths occurring in the hospital setting—or while on hospital property—is much sparser.

The database of deaths from the Harris County Institute of Forensic Sciences was searched from 2014 to 2019 using “hospital” as a search term in both the injury address and injury place. Neonates discovered dead, or who remained comatose and eventually were pronounced brain dead following being found unresponsive while in the hospital, were also included.

Study results revealed that deaths from intracranial hemorrhage due to falls remain, by far, the most common cause of non-natural death in the hospital setting; such deaths included falls in hospital parking lots. Suicides, in this study, included both patients recently discharged from psychiatric inpatient settings and those who were not admitted at all, but simply committed suicide on hospital property. In the former case, deaths were usually attributable to jumping from heights, such as parking structures, while in the latter case, deaths typically resulted from gunshot wounds in the parking lot. In this study, recently discharged patients most often killed themselves within hours of their discharge. Homicide deaths due to gunshot wounds occurring inside a hospital were identified but were rare, as were work accidents. Deaths due to airway obstruction (i.e., choking on a food bolus) all occurred during mealtimes.

A steady trend across all five years of the study is accidental death due to acute illicit drug toxicity by admitted patients, the majority of whom were admitted for diagnoses unrelated to acute drug withdrawal or inpatient rehabilitation and brought the drugs with them. This current study suggests that new strategies to help combat illicit drug deaths while in the acute care setting would be beneficial to overall patient safety.

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#### Hospital Property, Patient Safety, Non-Natural Deaths



### H149 Autopsy Rates for Injury Deaths by Manner, Cause, and State in the United States From 2015 to 2017

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**Learning Overview:** After attending this presentation, attendees will better understand current trends in autopsy rates and characteristics of deaths in which an autopsy was performed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the characteristics of injury deaths that are most likely to prompt an autopsy examination as well as the variation in autopsy performance practice by state.

The accuracy of cause- and manner-of-death information is dependent on several factors, including whether the decedent had an autopsy examination.<sup>1,2</sup> This study describes variation in autopsy rates for injury deaths by manner of death, cause of death, and state in which the medicolegal death investigation was conducted and a death certificate was completed.

The National Vital Statistics System Detailed Mortality data for the years 2015 to 2017 were analyzed to determine the characteristics of decedents and circumstances of death when autopsies were performed.<sup>3</sup> The query was based on the death certificate item: "Was an autopsy performed?" Response categories on a standard death certificate are "yes," "no," and "unknown".<sup>4</sup> The percent of injury deaths with autopsy status known and reported as "yes" was calculated by age of the decedent, manner and cause of injury-related death, and state. Manner-of-death and cause-of-death categories were defined using the *International Classification of Disease, 10<sup>th</sup> edition (ICD-10) External Cause of Injury Matrix*.

In 2015–2017, 54.5% of injury deaths were autopsied; 43.7% were not autopsied; and 1.8% had an unknown autopsy status. Injury deaths represented 8.3% of all deaths. Of non-injury deaths, less than 4% were autopsied and 5.0% had an unknown autopsy status. For all deaths, 8.0% were autopsied and 4.8% had an unknown autopsy status.

Autopsy rates varied by manner of death with a range of rates between the states. Almost all homicides were autopsied (98.6%, range by state from 90.2% to 100%). In contrast, 54.4% of suicides were autopsied (range by state from 5.7% to 97.2%); 49.6% of unintentional (accidental) injury deaths were autopsied (13.9% to 72.1%); and 83.0% of injury deaths of undetermined manner were autopsied (46.2% to 100%).

Autopsy rates also varied by cause of death. For drug toxicity deaths (all manners), 76.8% were autopsied (23.1% to 97.3%). For motor vehicle deaths, including bicyclists and pedestrians (all manners), 49.5% were autopsied (4.2% to 93.8%).

Among all injury deaths, the percent autopsied was highest for those less than 1 year of age (95.3%) and lowest for those over 65 (22.0%).

Autopsy rates vary by cause and manner of death, age of decedent, and the state in which medicolegal death investigation was conducted and death certified. Variation by state likely reflects differences in state statutes, budgets, and office customs.

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#### Autopsy, Death, Injury



### H150 Radically Invasive Projectiles (R.I.P.s) and Other Unusual Ammunition: A Case Series

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**Learning Overview:** After attending this presentation, attendees will be familiar with the characteristics of the R.I.P. and will better recognize the radiographic and autopsy findings associated with various unusual ammunition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sharing cases of unusual ammunition experienced by forensic pathologists in an urban setting with an exceedingly high rate of gun violence.

The landscape of firearms and ammunition is ever-changing, with different styles of ammunition becoming more readily available and other styles falling in and out of favor. Since recovery and identification of bullets and bullet fragments is an integral part of any death involving a firearm, this presents a challenge for examiners encountering either long-defunct, unique rounds or novel, highly engineered ammunition.

G2 Research's R.I.P. bullets are one such brand of novel ammunition that is rapidly growing in popularity. R.I.P. bullets are solid copper projectiles into which eight trocar spires are machine carved. The trocar design reportedly increases stability while allowing the bullet to function like a saw and effectively pass through solid barriers, such as sheetrock, cinderblock, and metal. When the R.I.P. bullet strikes a soft target, however, the solid base penetrates deeply into the target while the trocar elements break off and bore outward into adjacent structures. The R.I.P. bullet is therefore designed to have the maximum stopping power of a hollow-point bullet with the collateral damage of a frangible projectile. At autopsy, the R.I.P. bullet's design results in atypical wound patterns and paths, which can be difficult to interpret, especially in the setting of multiple projectiles. Awareness of the extremely sharp trocar fragments is particularly important as they can be a hazard to the examiner and the technician performing the case.

While basic knowledge of ammunition is essential for the forensic pathologist, those practicing in an urban setting are increasingly exposed to unique ammunition and need to become more attuned to its distinct characteristics. In most cases, forensic pathologists are initially confronted with these challenging bullets when radiographic imaging is performed, revealing foreign bodies with unusual shapes and contours, sometimes mimicking clothing or personal belongings. Familiarity with the characteristics of unique ammunition, such as the R.I.P. bullet, is indispensable when confronted with one of these challenging cases.

This presentation describes the unique radiographic and autopsy findings in several cases encountered at the State of Maryland Office of the Chief Medical Examiner involving unusual ammunition including: G2 Research's Radically Invasive Projectiles, Taurus Judge .410 bore shot shells, MagSafe® ammunition, PolyCase® ARX composite ammunition, Expanding Full Metal Jacket bullets, #4 buckshot, conical pellet shot shells, black powder rounds, and snake shot ammunition.

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#### **Ammunition, R.I.P., Ballistics**



### H151 Severe Hemorrhagic Retinopathy and Retinoschisis Associated With Hypoxic Ischemic Brain Injury and Coagulopathy Due to Aspiration of Popcorn Kernels

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**Learning Overview:** After attending this presentation, attendees will learn that extensive Retinal Hemorrhages (RHs) and retinoschisis can occur in young children who have non-traumatic hypoxic ischemic brain, cerebral edema, and coagulopathy due to aspiration.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reinforcing the necessity for routine postmortem ocular examinations of children with non-traumatic, hypoxic ischemic brain injury, cerebral edema, and coagulopathy.

Some authors suggest that non-traumatic hypoxic-ischemic brain injury with cerebral edema and coagulopathy does not cause severe extensive RHs or retinoschisis. Presented here is a case of a toddler who aspirated unpopped popcorn kernels and experienced a cardiopulmonary arrest. Return of spontaneous circulation occurred after >60 minutes of Cardiopulmonary Resuscitation (CPR) after which she developed hypoxic ischemic brain injury and coagulopathy. The autopsy findings of this case refute the purported specificity of extensive RHs and retinoschisis in young children for abusive head trauma (shaken baby syndrome).

**Case Report:** A 19-month-old toddler experienced a choking episode at home. Her father gave back blows while her mother called 911. When paramedics arrived, she was unresponsive and in pulseless electrical activity. She received over 30 minutes of CPR with intermittent return of spontaneous circulation. During intubation in the emergency department, food debris and unpopped popcorn kernels were removed from her airway. After an additional 30 minutes of CPR, a return of spontaneous circulation was achieved. Following admission to the Pediatric Intensive Care Unit, she developed coagulopathy with a prothrombin time (>90 seconds), partial thromboplastin time of 174.2 seconds, a fibrinogen of 50mg/dL, and a platelet count of  $171 \times 10^3/\mu\text{L}$ . Given the extent of neurological injury from prolonged cardiac arrest, her family decided to withdraw medical care. She died 15 hours after the choking episode. No clinical fundal examination was recorded in the medical record.

Postmortem indirect ophthalmoscopy revealed bilateral extensive RHs extending past the equators with a large subinternal limiting membrane hemorrhagic cyst (schisis cavity) adjacent to the right inferior temporal vascular arcade. Both fundi had numerous splinter, flame-shaped, dot, and blot RHs plus small wisps of vitreous hemorrhage. An unpopped popcorn kernel occluded her right mainstem bronchus and another popcorn kernel was removed from her oropharynx. Her brain was markedly edematous and contained scattered hypoxic-ischemic neurons. Her retinae exhibited numerous RHs involving the nerve fiber, ganglion cell, inner plexiform, inner nuclear, outer plexiform, and outer nuclear layers; the RHs extended to the ora serrata. The large hemorrhagic cyst over the right fundus consisted of separation of the internal limiting membrane (retinoschisis) with subjacent extravasated blood. Focal intraneural hemorrhage involved the lower cervical spinal cord nerve roots.

This case describes extensive hemorrhagic retinopathy extending to the ora serrata and retinoschisis associated with hypoxic-ischemic brain injury, cerebral edema, and coagulopathy following prolonged resuscitation due to a cardiopulmonary arrest from aspirated popcorn kernels. This study urges caution in interpreting ocular findings out of context since extensive RHs and retinoschisis have been considered diagnostically specific for abusive head trauma (shaken baby syndrome). This case highlights that these ocular findings are not diagnostically specific for abusive head trauma and emphasizes the importance of consistent and thorough postmortem ocular examination in young children with non-traumatic cerebral edema and coagulopathy.

#### Retinal Hemorrhages, Retinoschisis, Hypoxic Ischemic Brain Injury



### H152 A Tale of Two Recreational Mummification Bondage Cases: Undetermined Versus Homicide

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**Learning Overview:** After attending this presentation, attendees will better understand the importance of scene investigation in certifying deaths related to recreational mummification bondage.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the importance of a thorough scene investigation with implications on the manner of death certified in recreational mummification bondage-related deaths.

**Case 1:** A 48-year-old man experienced in bondage sessions and body wraps was found in a residential basement converted to a Bondage & Discipline, Dominance & Submission, Sadism & Masochism (BDSM) -style dungeon after his partner realized he was not responding appropriately. The decedent and partner had known each other for seven months and had regular bondage sessions at the location, the last session two weeks prior to death. According to the partner, the decedent was covered from head to toe by plastic wrap and gaffer's tape with small breathing holes at the mouth and nose. At the scene investigation, the decedent was found nude, supine, with a locked metal chain around his neck, and a penile chastity cage. A trash can in the corner of the room contained plastic wrap and red gaffer's tape that the partner had removed once the decedent was unresponsive. In this instance, video and photos of prior events were not collected into evidence, and reportedly there were no videos or photographs of the event in question. Injuries seen at autopsy included contusions and edema of the genitals, consistent with the male chastity belt, and superficial erythematous horizontal lines on the lower extremities, consistent with the description of being wrapped in plastic and tape. Mild pulmonary congestion and edema were present, and no significant natural disease was seen anatomically. Toxicology studies revealed a low (11mcg/mL) concentration of Gamma Hydroxybutyrate (GHB) in the decedent's blood. Both the GHB, a respiratory depressant, and the restriction of the chest caused by mummification were thought to contribute to an increased risk for respiratory distress. The cause of death was sudden death during recreational mummification bondage due to an undetermined cause after autopsy and toxicologic analysis, and the manner was undetermined.

**Case 2:** A 27-year-old woman was found unresponsive in a hotel room; cellophane, white duct tape, and pink duct tape were in a trash can in the room. Four videos showing the encounter from varying angles were recovered at the scene, depicting the suspect wrapping and striking the decedent; only the decedent's breasts and nose were exposed. The video shows the suspect occlude the decedent's exposed nose, at which time she begins markedly struggling against the restraining tape. The woman becomes unconscious but remains alive, until the suspect removes the tape from the waist down and begins sexual intercourse. After a total elapsed occlusion time of approximately 17 minutes, the man removes the tape from the cyanotic nose, releasing tan-brown fluid from the nares. At autopsy, there were abrasions of the face, neck, left breast, and left thigh; contusions and petechiae of the right and left breasts with patterned contusions of the left breast. Internally, there were scalp contusions and hemorrhages in the neck muscles, though there was no fatal blunt trauma. There was complete occlusion by gastric contents of the right and left mainstem bronchi with near complete occlusion by gastric contents at the level of the vocal cords. No significant natural disease and no lethal drug or alcohol toxicity were present. The cause of death was asphyxia due to smothering and the manner was homicide.

This report of two recreational mummification bondage-related deaths highlights the importance of scene investigation with collection of evidence, including video footage, photos, and detailed histories. Once deemed unresponsive, the bondage tape is often removed during attempts at resuscitation, and the evidence of the events may be lost unless there is a reliable witness or form of event documentation. Without definitive scene evidence, as the findings at autopsy are non-specific, a cause and manner of death should be carefully considered, as an "act of mutual consent" could be taken out of context.

#### **Mummification, Bondage, Asphyxia**



### H153 Two Firework Fatalities in North and Central Texas

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**Learning Overview:** The goal of this presentation is to educate medical examiners and forensic investigators of the range of findings in firework fatalities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating those involved in investigation of potential firework deaths regarding the range of possible findings.

Upon completion of this presentation, viewers will have gained exposure to rare instances of fireworks fatalities, readily recognize patterns of external injury, and list potential causes of death in these cases. Cases examined by the Tarrant County Medical Examiner's Office, Fort Worth, TX, will be discussed in depth.

Independence Day fireworks displays are a tradition in the United States. Although most accidents involving fireworks result in blast or thermal injuries of varying severity, two cases of accidental death investigated at the Tarrant County Medical Examiner's Office showed additional findings of interest.

In July of 2019, a 19-year-old male partook in a deadly game of fireworks war with friends that resulted in his sudden death. The subject ignited his weapon of choice, and an unexpected explosion caused a severe blast injury to his left hand. In addition to the blast injury of the hand, external examination revealed superficial wounds of the central chest, believed initially by some to be attributable to the LUCAS® chest compression device used during resuscitative efforts. Closer inspection revealed a thermal injury in a circular pattern superior to the resuscitation injury. Internal examination exposed fracture with associated hemorrhage of the sternum. Additionally, there was 550mL of blood in the pericardial sac, rupture of the anterior wall of the left ventricle, rupture of the posterior wall of the left ventricle involving the aortic valve, and rupture of the interventricular septum. The cause of death was blunt force injury of the chest due to fireworks explosion. The manner of death was ruled as accident.

A police vehicle dash camera caught the explosion on film; however, it was too far away to show details of the incident. Examination of the scene revealed a cylindrical pyrotechnic device status post-combustion with the clay seal separated. This finding corroborated the witness accounts indicating that he was holding the firework up to his chest, pointing it outward at his friend when it exploded, ultimately providing the mechanism for the pattern of injury on the victim's chest.

In July of 2014, a 51-year-old male was killed in a fireworks incident that resulted in three additional injuries. In preparation for a Kiwanis® event, seven people were unloading fireworks and placing electric matches into six-inch fireworks shells at the rear of a trailer. During the process, a shell became live and detonated. The shell caused other shells to ignite and spread to the open trailer, igniting the majority of the fireworks still in the trailer. The fire then spread to other nearby combustibles, two vehicles, and the trailer. The trailer showed no signs of detonation; instead it had melting of the metal frame, siding and structural components. Investigation could not exclude accidental activation of a defective electric match, improper handling of electric matches or fireworks, or accidental activation of the internal firework fuse by friction caused by metal scissors during the process of installing the electric match.

The decedent was examined at the office and displayed extensive thermal injury, cherry-red discoloration of soft tissues and viscera, and soot in the airways with a carbon monoxide level of 16%. In addition, there was penetration of the neck by a foreign object that injured the internal and external jugular veins at the brachiocephalic vein. There were fragments of laminated pressed cardboard and clear plastic along the wound track. The cause of death was ruled as inhalation of smoke and penetrating injury of the neck due to fireworks explosion. The manner of death was ruled as accident.

In the 2018 Fireworks Annual Report, the United States Consumer Product Safety Commission (CPSC) reported that most fireworks injuries and fatalities seen in emergency rooms occur during the month surrounding July 4.<sup>1</sup> Hospital emergency departments treated approximately 9,100 fireworks injuries. An estimated 62% (5,600) of those injuries treated occurred during the month surrounding Independence Day. The most frequent injuries treated involved the hands and fingers (28%), followed by injuries of the legs (24%) and injuries of the eyes (19%). Only five deaths attributed to fireworks explosions were reported at the time of the CPSC's Annual Report.<sup>1</sup>

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- <sup>1</sup> United States of America Consumer Product Safety Commission. 2018 Fireworks Annual Report. *Fireworks-Related Deaths, Emergency Department-Treated Injuries, and Enforcement Activities During 2018*.

#### Firework, Explosion, Fatality





### H154 “Who Dismembers Mama?”

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**Learning Overview:** After attending this presentation, attendees will be familiar with cases involving dismemberment, including the different modes of dismemberment, patterns used to classify them, causes of death, and concealment locations in a large metropolitan area.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by familiarizing death investigators with different patterns of, and implements used in, dismemberment.

Dismemberment is uncommon and is typically performed to hinder identification and/or facilitate the disposal of the decedent. Dismemberment poses specific challenges to the forensic community, including not only in identification of remains, but also in the determination of cause and manner of death. There are different classification systems that describe dismemberment. One classification system encompasses postmortem mutilation and dismemberment. This classification emphasizes the psychology of the perpetrator and describes the postmortem mutilation/dismemberment as motivated by: (1) defensive (disposal of the remains and/or hindering identification), (2) aggressive (outrage and involves mutilation of face and genitals), (3) offensive (necrophilia urge and sexual sadism), and (4) necromanic (necrophilia and retaining a body part as a trophy). Understanding a perpetrator’s motive is useful, but knowledge of specific patterns of dismemberment is also very helpful. Dismemberment patterns can be described as localized (removal of body parts to hinder identification) or generalized (segmenting the body for disposal). Both localized and generalized dismemberment are further subdivided by the method of dismemberment. Patterns can also be described as involving disarticulation, transection by sawing or chopping, or a combination of the two.

In this study, the Cook County Medical Examiner’s Office records were retrospectively searched from 1991 to 2018 to identify cases in which the manner of death was homicide or undetermined, and included the following terms: dismemberment, decapitation, disarticulation, and unidentified bones. Seventeen cases were identified that met these criteria. The dismemberment pattern, cause of death, recovery location, demographic data, type of implements, and other postmortem artifacts were recorded.

The manner of death was homicide in 94% of the cases. An undetermined manner accounted for the remaining 6% of the cases. Female victims accounted for 53% of deaths and their ages ranged from 3 to 81 years. Of the female victims, 44% were black, 44% were White, and the race could not be determined in one case. The cause of death for female victims ranged from sharp force injuries to blunt force injuries with each accounting for 11.1% of the causes of death, for a total of 22.2%. Homicide by unspecified means accounted for 66.7% of the cases. The cause and manner of death was undetermined in 11.1% of the cases.

Male victims made up 47% of the cases, with ages ranging from 2 to 70 years. Of the male victims, 37.5% were Black and 62.5% were White. Cause of death was blunt force injuries in 25% cases. Decapitation, sharp force injury, head trauma, and gunshot wound to the head comprised the remainder of the male victims’ cause of death when one was identified. Each of these four cases accounted for 12.5% for a total of 50%. Homicide by unspecified means made up the remaining 25%.

The entire body was recovered in 52.9% of the cases (i.e., 29.4% of the female victims and 23.5% of the male victims.) Dismemberment and removal-disposal/concealment was an element in 94% of the cases. In only one case was there an element of display rather than concealment. No correlation was found between the cause of death, gender, age, race, or type of dismemberment pattern among the female and male victims. Implements used in dismemberment were identified in 64.7% of the cases. No correlation was identified between cause of death and type of dismemberment/transection pattern (i.e., transection by saw, by chop, or by disarticulation).

Transection by saw predominated. Disarticulation, which appears to be a logical mode of dismemberment, was rare in this and other studies. One can only speculate as to the possible reasons for this; although disarticulation requires less force, it may be more time-consuming and require an understanding of anatomy that an average perpetrator may lack. Additionally, the prevalence of hardware stores and the availability of tools that can be used to dismember a body make transection easier. Recognition of this fact in at least one of the cases reviewed permitted the implements used to be traced to purchases made at a hardware store with subsequent identification of the perpetrators.

### Dismemberment, Disarticulation, Transection



## H155 Pull It Out or Leave It In? Foreign Bodies Discovered During Medicolegal Death Investigation

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**Learning Overview:** The goal of this presentation is to employ case examples to emphasize the importance of applying a multidisciplinary approach in death investigations in which foreign bodies are present as well as to highlight the potential confusion caused when implements are removed and not properly documented prior to the involvement of the Medical Examiner's or Coroner's Office (MEO).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the vastly variable scenarios death investigators experience with the goal of promoting the importance of multiagency communication. The intended result is to instill within attendees a level of caution and consideration when responding to death scenes where foreign objects are present.

Although the characteristics of penetrating wounds can support a determination of what implement caused it, leaving impaled foreign objects *in situ* obviously decreases any potential confusion regarding the implement that can arise. Through a review of six case examples, this presentation will address a series of four considerations from the MEO perspective for outside agencies to review when handling cases with foreign bodies found impaled or inserted into human remains, including during first response. More specifically, the question of scene and responder safety, the importance of documentation, the value in keeping items *in situ* to avoid confounding confusion, and how all of these items come together to support considerations for cause and manner of death will be reviewed.<sup>1-6</sup>

**Case 1:** A 28-year-old male and his brother were in a verbal altercation during which the decedent's brother stabbed the hilt of a handmade metal sword into the decedent's head.

**Case 2:** A 63-year-old female was found deceased in her yard with polytrauma, including a knife stuck in her buttock.

**Case 3:** A 46-year-old male was witnessed to go unresponsive at home during sexual intercourse. An anal plug had been inserted with consent by his wife and remained *in situ* upon the arrival of the MEO.

**Case 4:** A 23-year-old female was stabbed multiple times by her Significant Other (SO). The SO reported that she'd been seated, he'd been standing over her and holding their infant at the time of the stabbing. The knife had been left in the decedent's body by the suspect. However, upon arrival, medics reported noting signs of life so they removed the knife and set it aside to facilitate resuscitative efforts.

**Case 5:** A 32-year-old male was found unresponsive at a bus stop lying in a fetal position. The circumstances surrounding his being there were unclear until the decedent was rolled during his external exam and a syringe was found *in situ* in his arm.

**Case 6:** A 41-year-old male driving a stolen vehicle at slow speeds lost control of the vehicle, which then struck a chain link fence and a utility pole. The top rail of the fence was displaced and impaled the decedent through the mouth and exited through the back of the head.

Reconstruction of injury patterns and determining their instrument of creation is imperative to determining cause and manner of death in cases of penetrating trauma. A thorough death scene investigation includes a multiagency discussion, especially when attempts at lifesaving efforts were made and the original scene was disturbed. In this presentation, it will be suggested that the best way to avoid the potential for confusion in death investigations is to have a set protocol when interacting with first responder agencies in cases where foreign bodies are present.

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### Foreign Objects, Impale, Postmortem Artifact



### **H156 The Importance of Histopathology in Cases of Sudden Death Due to Alleged Electrocution With Inconclusive Autopsy Findings: A Report of Three Cases**

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**Learning Overview:** After attending this presentation, attendees will understand the importance of histopathological examination of suspected skin wounds and the heart in cases of sudden death due to alleged electrocution where autopsy findings are inconclusive.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community as attendees will understand the importance of histopathological examination of suspected skin wounds and the heart in sudden death due to alleged electrocution, especially in those cases where the autopsy findings of electrocution are not clear and specific.

The pattern of injuries noted in high-voltage electrocutions is appreciable during autopsy examination. But such clear-cut findings are not found in many cases of low-voltage electrocution. Though joule burns are classically described as the entry wound feature of low-voltage electrocution, they are not seen in many cases. In such cases, doubt may persist in the mind of the forensic pathologist regarding whether to designate them as electrocution entry wounds or not. Similarly, the exit wound may either be absent or else not clearly appreciable via naked eye examination in many cases. In all such cases, histopathologic examination of a suspected entry and exit wound is essential. Ventricular fibrillation is the most common cause of cardiac arrest in electrocution. This most typically occurs when current enters through the right hand and exits through the feet. Hence, histopathologic examination of the heart is also required where death occurs immediately after electrocution.

**Case 1:** A do-it-yourself electrician was repairing a faulty motor pump meant to drain water from a swimming pool. He was accidentally electrocuted and died within few minutes. Suspected electrocution entry and exit wounds were noticed on the right index finger and the heel of the right foot. Tissues from these sites and samples from control sites and the heart were submitted for histopathology. Tissues from the entry and exit wounds showed microscopic features of electrocution. The heart showed features of myofibre breakup. Control site samples were histologically within normal limits.

**Case 2:** A brick kiln laborer was accidentally electrocuted when the metallic rod he was using came into contact with overhead electric wires. Suspected electrocution contact wounds were noticed on the left thumb and the adjoining hypothenar area. No exit wounds were appreciated. Tissues from the suspected entry site and heart were submitted for histopathology. Features of electrocution were observed on microscopic examination of both.

**Case 3:** A welder was electrocuted while at work. A suspected electrocution contact wound in the form of skin reddening was noticed in the hypothenar area of the right hand. A suspected exit wound in the form of a crater 0.3cm in diameter was noticed on the dorsal aspect of the medial right foot about 15cm behind the tip of the great toe. Tissues from the suspected entry wound showed intra-epidermal separation with a dense eosinophilic appearance of the keratin layer. The suspected exit wound had features of sub-epidermal separation and coagulative necrosis of both the epidermis and dermis. A section of the heart showed features of myofibre breakup.

These three cases will be reviewed individually. In alleged electrocution cases, where death is sudden and autopsy findings are unclear, histopathologic examination of the heart and the suspected skin wounds should be mandatory. A final opinion regarding cause of death can be given after collectively considering the history, autopsy findings, circumstantial evidences, and the histopathologic findings.

#### **Alleged Electrocution, Suspected Skin Wounds, Heart Histopathology**



## H157 Developing Novel Microbial Community Metrics for Predicting Manner of Death (MOD)

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**Learning Overview:** After attending this presentation, attendees will better understand that the community of microbes after death, or the postmortem microbiome, has further forensic utility than postmortem interval estimation. Attendees will see how beta-dispersion profiles, a novel application of the “Anna Karenina Principle,” have predictive power for MOD determination due to the insight postmortem microbiomes provide of antemortem health conditions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic scientific community by presenting how specific postmortem microbiome community metrics may provide a novel tool for MOD determination.

Microbiomes have an important functional role in a host's life, influencing health, development, and disease susceptibility, among many other aspects of human well-being.<sup>1</sup> Microbes also play an important functional role in decomposition after death.<sup>2</sup> Postmortem microbiomes are known to undergo changes specific to anatomical body sites, and follow successional patterns that track the postmortem interval.<sup>2-4</sup> The postmortem microbiome has potential as a tool for postmortem interval estimation. However, the microbial community has additional applications, due to its ability to reflect antemortem health conditions, such as disease state.<sup>3</sup>

As the antemortem microbiome is consistent with the postmortem microbiome within 24 hours after death, microbial community metrics could potentially reveal associations with MOD.<sup>3</sup> The postmortem microbiome is hypothesized to vary depending on a decedent's antemortem health condition related to lifestyle stressors. Stressors that may include, but are not limited to, heart disease, drug/alcohol abuse, and/or a high anxiety lifestyle indicative of certain manners of death (i.e., homicide). This community structure reflected in the postmortem microbiome is thought to vary with the conceptual context of the Anna Karenina Principle. The Anna Karenina Principle asserts that microbiomes of unhealthy individuals have increased beta-dispersion (i.e., microbial community variability) than those of healthy individuals (less variability).<sup>1</sup> The Anna Karenina Principle has shown shifts in microbial communities in living individuals associated with obesity, infection, and smoking.<sup>1,5</sup> Due to the postmortem microbiome reflection of antemortem conditions, the Anna Karenina Principle provides an important conceptual context for quantifying microbial signatures and developing metrics or profiles associated with MOD determinations.

This study sought to determine how beta-dispersion profiles, or beta-dispersion differences associated with certain MODs or antemortem conditions, could potentially be used to confirm MOD assessments—in other words, answering the question: is a postmortem microbiome profile consistent with an MOD assigned in a death investigation? To determine if postmortem microbiomes could be used to answer such a question, 188 United States routine autopsy cases with samples taken from five anatomical sites to test associations of MOD and antemortem conditions, including heart disease, drug use, and gunshot deaths, were reviewed. Previously published amplicon sequence data were analyzed using QIIME2™ (v.2018.11) and beta-dispersion metrics were assessed in R using the *vegan* library to statistically model microbiomes with different MODs and test if the microbial communities varied with the Anna Karenina Principle.<sup>3</sup>

Applying the Anna Karenina Principle to postmortem microbiome beta dispersion profiles resulted in different degrees of dysbiosis and were associated with different MODs, with predictive accuracy that depended on sample type. Preliminary results showed that beta-dispersion differed among body sites, with rectum communities having the highest beta-dispersion, while eyes had the lowest. Across all body sites, natural deaths and suicides had overall higher beta-dispersion than homicides. Also, cases with cardiovascular disease-related deaths had significantly higher beta-dispersion than gunshot or drug-related deaths, while non-violent deaths had higher beta-dispersion than violent deaths. Overall, increased beta-dispersion is more prevalent in MODs associated with disease states (cardiovascular disease and mental health issues) compared to homicides and drug-related deaths. Previous research has confirmed reduced beta-diversity in violent deaths as well as reduced green space access.<sup>3,6</sup> Therefore, the current results support the hypothesis that antemortem conditions are associated with postmortem microbiome beta-dispersion profiles and that such data could have potential utility in death investigations by providing corroborating, or contradicting, microbial evidence of MOD as determined by a reflection of antemortem lifestyle. However, additional studies are needed to confirm and expand these findings.

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### Forensic Microbiology, Postmortem Microbiome, Manner of Death Determination

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### H158 The Impact of Confinement in Vehicle Trunks on Decomposition and Entomological Colonization of Carcasses

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**Learning Overview:** The goal of this presentation is to inform attendees about the impact on decomposition and insect colonization when confining a carcass inside a vehicle trunk in comparison with exposed carcasses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the differences in entomological colonization and decomposition and delay in colonization caused by confining a carcass inside a vehicle trunk, a common way of disposing of a body. It is important that these differences are understood by forensic investigators, particularly forensic entomologists.

In order to investigate the impact of confinement in a car trunk on decomposition and insect colonization of carcasses, three freshly killed pig (*Sus scrofa domesticus* Erxleben) carcasses were placed individually in the trunks of older-model cars and deployed in a forested area in the southwestern region of British Columbia, Canada (Coastal Western Hemlock Biogeoclimatic Zone, Dry Maritime Subzone), together with three freshly killed similar carcasses that were exposed in protective cages in the same forest. The cages prevented bears and other vertebrates from removing the carrion but allowed insect access. Decomposition rate and insect colonization of all carcasses was examined twice a week for four weeks. At each collection time, the cars were covered with an extensive plastic sheet to prevent egress and ingress of insects while the researcher entered the vehicle for collection.

The exposed carcasses were colonized immediately by *Calliphora latifrons* (Hough) and *C. vomitoria* (Linnaeus) (Diptera: Calliphoridae), followed by *Lucilia illustris* (Meigen), *Phormia regina* (Meigen), and *Protophormia terraenovae* (R.-D.). There was a delay of three to six days before the confined carcasses were colonized, first by *P. regina*, followed by *Pr. terraenovae*. These species remained the sole blow fly species on the confined carcasses. Temperatures were greatly increased inside the vehicles. Despite the delay in colonization, decomposition progressed much more rapidly in two of the confined carcasses in comparison with the exposed carcasses due to the greatly increased temperatures inside the vehicles, with the complete skeletonization of two of the confined carcasses between 9 and 13 days after death. One confined carcass was an anomaly, attracting many fewer insects, supporting fewer larval calliphorids, and decomposing much more slowly than other carcasses, despite similarly increased temperatures. It was later discovered that the vehicle in which this carcass was confined had a solid metal fire shield between the passenger area and the trunk, which served to reduce insect access and the release of odors. These data may be extremely valuable when analyzing cadavers found inside vehicle trunks.

#### Entomology, Confinement, Decomposition



## H159 Estimating the Postmortem Submersion Interval (PMSI) From the Microbiome of Bone in Lacustrine and Riverine Environments in Virginia

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**Learning Overview:** After attending this presentation, attendees will understand how different aquatic environments (i.e., freshwater lake and freshwater river) impact microbial community changes across Accumulated Degree Days (ADD).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the potential use of universal microbial succession on submerged skeletal remains to predict long-term PMSI.

Water-related deaths, criminal activity, and mass disasters occurring over or in water (e.g., boating accidents, ferry disasters, commercial plane crashes, and natural disasters) result in thousands of deaths every year. In Virginia alone, the Office of the Chief Medical Examiner reported that death by drowning (asphyxia) accounted for 91 accidental deaths in 2017.<sup>1</sup> Bodies recovered from aquatic environments are exposed to diverse and changing environmental conditions (i.e., scavengers, microorganisms, temperature, pH, current, etc.). Furthermore, microorganisms, such as bacteria, algae, and fungi, participate in the decomposition of human remains in an aquatic environment. With recent advancements in technology, studies have identified microorganisms as potential estimators of time since submersion (PMSI).<sup>2,3</sup> Unfortunately, a reliable and universal method for determining long- and short-term PMSI has yet to be developed.

To examine the impact of aquatic environments on microbial succession, a freshwater river (James River, Charles City, VA) and lake (Henleys Lake, White Hall, VA) were selected as field sites. Work at Henleys Lake and the James River occurred between November 2016–June 2018 and November 2017–November 2018, respectively. For both sites, fresh pig (*Sus scrofa*) bones (scapulae and ribs) were submerged in cages that were attached to a flotation device or wharf. Waterproof temperature loggers and a YSI Sonde were used to record environmental parameters. Every 250 ADD, five scapulae, five ribs and 500ml of water were collected and stored at either -80°C or 4°C. Sample processing included filtration on 0.22µm filters for water samples and pulverization via liquid nitrogen in a mortar and pestle for bone samples. Both filters and powder underwent DNA extraction and purification using ChargeSwitch® gDNA Plant Kit and DNeasy® PowerClean Pro Cleanup Kit, if necessary. Library preparation and sequencing-by-synthesis of microbial 16S recombinant DNA (rDNA) variable region 4 followed the established Kozich et al. and was performed via Illumina's® MiSeq® 2X300 paired-end sequencing.<sup>4</sup> Data analysis and visualization was completed using the MiSeq® mothur SOP, mothur version 1.35.9, and R studio.<sup>5</sup>

Preliminary analysis suggests that there are significant differences between locations and among sample types at each location. In regard to relative abundance, changes were observed in rib and scapula samples; furthermore, scapula and rib samples at both locations shared Firmicutes and Proteobacteria as the two dominant phyla across time; however, the remaining taxa varied. Both locations also demonstrated significant differences in ordinated space (Bray-Curtis Operational Taxonomic Units [OUT] beta-diversity) for each sample type across ADD. Additionally, curvilinear relationships between shannon diversity and ADD were observed for scapula and ribs at both locations. The aforementioned results suggest that there may be enough similarities between locations to create a long-term PMSI model for Virginia.

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### PMSI, Aquatic Environments, Bone Microbiome



## H160 Evaluating the Nearest Weather Station as the Best Representation of Scene Temperature in Forensic Entomology Casework

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**Learning Overview:** After attending this presentation, attendees will gain appreciation of the difficulty of selecting appropriate weather station temperature data to represent insect development conditions at scenes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting data on long-term comparison between temperatures as encountered at scenes during medicolegal death investigations and the nearest weather stations. These data will also attempt to answer the question of which weather station(s) and temperature data may provide more accurate representation of scene conditions, which directly influence insect development.

Insects are poikilothermic, capable of surviving wide temperature shifts and acquiring much of the heat energy required to complete development from their environment. Forensic entomology depends upon this relationship between insect development and environmental temperature to estimate insect age.<sup>1</sup> Assuming that the insects colonized the deceased following death, the estimated insect age may correspond to the Time Of Colonization (TOC) and a measure of the Postmortem Interval (PMI), depending upon scene conditions and the quality of the underlying data. Recommended practices for forensic entomology rely on collection of weather data from the nearest weather station to feed the calculations underlying insect age estimates.<sup>2</sup> The use of the nearest weather station has been widely assumed to be the most accurate temperature data for a given scene. At outdoor scenes, weather station temperature data appears to be a reasonable surrogate for historical scene temperatures in many scene scenarios. How well the scene temperatures are reflected by the nearest weather station can be evaluated with longer-term scene temperature collections for a calculated comparison and adjustment of the weather station data.<sup>3</sup> However, indoor active temperature modification, away from the nearest weather station temperatures, may be occurring during decomposition and insect development.

This study utilized button temperature sensors to examine the long-term differences between fixed locations, hypothetical scene locations, and the nearest weather stations (Airports: George Bush Intercontinental Airport [IAH], William P. Hobby Airport [HOU], Sugar Land Regional Airport [SGR], Maicai Airport [MCJ], and Conroe-North Houston Regional Airport [CXO]). Furthermore, the study was designed to examine one of the most commonly encountered temperature-modifying effects in medicolegal casework, the effects of being indoors with and without air conditioning/heat. Button temperature sensors recorded hourly temperatures for one calendar year indoors w/air conditioning/heat and outdoors in Houston, TX, and at a location in Conroe, TX, indoors w/out temperature modification and outdoors. These data were then compared to hourly temperature data from five local weather stations at the same times. Regression was used to evaluate the relationship between indoor and outdoor temperatures with the different nearby weather stations.

The results of these long-term evaluations revealed that local outdoor temperatures were informative predictors of indoor temperatures, even in the presence of indoor climate control. Furthermore, the nearest weather station was not always the best predictor of the hypothetical scene temperatures. The results of model validation and evaluation of additional hypothetical scene locations will be presented. Additionally, the potential for directly relating these results to insect development will be discussed.

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### Insect, Temperature, Weather Station

## H161 The Impact of Insect Exclusion on Eukaryotic Community Succession on Porcine Remains

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**Learning Overview:** After attending this presentation, attendees will better understand the eukaryotic community associated with porcine cadavers and how the eukaryotic community changes with time in the presence and absence of insects.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving Postmortem Interval (PMI) estimation methods based on these succession patterns.

PMI estimation is a vital part of forensic investigations, yet determination methods for long-term PMI are not reliable. Currently, microbial signatures are being investigated, specifically concentrating on the bacterial or eukaryotic succession patterns as PMI predictors.<sup>1</sup> Insect accessibility to a carrion has long been established as a driving force of the decomposition process.<sup>2,3</sup> Understanding which microbiota are released into these systems, as well as their origins, can aid forensic scientists in establishing robust and reliable PMI estimation methods. While forensic entomology has been useful for long-term PMI estimation, this method does not reflect situations in which remains are not immediately accessible to insects.<sup>4,5</sup>

This study characterized the eukaryotic community associated with porcine remains that were either exposed ( $n=6$ ) or excluded ( $n=6$ ) from insect access, using 18S recombinant DNA (rDNA) MiSeq<sup>®</sup> sequencing. Skin swabs were collected from each cadaver from the lateral thoracic or lateral abdominal regions starting immediately after euthanization, daily for the first 5 days, and weekly after this up to day 61 (1,703 Accumulated Degree Days [ADD]). DNA was extracted using the organic Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method followed by dual-index 18S rDNA MiSeq<sup>®</sup> sequencing on MiSeq<sup>®</sup> FGx platform using MiSeq<sup>®</sup> reagent kit v2 (500 cycles).<sup>5,6</sup> Sequences were analyzed using mothur version 1.39.5 and using SILVA (release 132) as reference data for eukaryotic classification.<sup>7</sup> Statistical analysis was performed using R version 3.5.0.<sup>8</sup>

An Analysis of Molecular Variance (AMOVA) suggested that there is a significant difference between the insect access and insect exclusion groups. However, the major phyla (>1%) differed only in their relative abundance. At the family level, similar patterns in top taxa can be seen. In both the access and exclusion groups, there is an increase in relative abundance of Rhabditida around 209 ADD/7 Days and an increase in the abundance of Dipodascaceae immediately after 448 ADD/15 Days. During the later stages of decomposition, the exclusion group showed much higher relative abundances of Filobasidiales and Trichosporonaceae, whereas the access group showed much higher relative abundances of Diptera, Coleoptera (as expected), and Ascomycota.

In conclusion, this study provides information on eukaryotes associated with porcine remains under both insect access and exclusion conditions. Here, succession patterns in both groups can be useful as predictors of the PMI. Utilizing this information in combination with prediction modeling may aid the forensic science community in filling gaps of extended PMI estimation methods, decreasing the overall rate of erroneous results.

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### Insect Exclusion, 18S rDNA, Postmortem Interval





### H162 Understanding the Role of the Thanatomicrobiota in the Decay of “Reproductive Organs” in Human Decomposition

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**Learning Overview:** After attending this presentation, attendees will understand how to use 16S ribosomal RNA (rRNA) amplicon sequencing analyses to characterize the thanatomicrobiota of reproductive organs from actual cadavers in criminal cases (e.g., homicide, suicide, and overdose). Specifically, attendees will learn methods to assess the microbial diversity after death using cases with Postmortem Intervals (PMIs) between 3.5 and 240 hours.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by revealing the specific bacterial signatures associated with the uterus and prostate of cadavers with different manners of death. These signatures could help to improve trace evidence regarding characteristics of manner of death for criminal cases.

Human organs decompose at different rates and in different ways. For example, human prostate glands and uteri are the last internal organs to deteriorate during putrefaction. However, the reason for this phenomenon has not been elucidated. To determine whether the bacteria associated with these organs differ from other organs, and to determine whether the taxonomic signature is associated with the PMI, this study applied 16S rRNA amplicon sequencing to tissues associated with 21 prostate glands and 13 uteri collected at autopsy from criminal casework cadavers. The 16S rRNA V4 region was amplified and sequenced from each sample, and non-parametric statistics were used to determine the resulting microbiota profile and its association with cadaver characteristics. Both the uterus and prostate had a significantly greater alpha diversity compared to other organs, as well as maintaining a significantly different microbial composition (beta diversity) as determined by unweighted UniFrac. The prostate was significantly enriched for two 16S rRNA Absolute Sequence Variants (ASVs) associated with the Bacteroidia, one in the family Comamonadaceae (genus *Limnohabitans*) and another in the family Oxalobacteraceae. Uterine tissues were enriched for only two ASVs, including a single ASV in the class Bacilli (family Lactobacillaceae, genus *Lactobacillus*) and a single ASV in the class Gammaproteobacteria (family Enterobacteriaceae, unknown genus). Prostate tissues had a significant underrepresentation of 4C0d-2 ASV (order MLE1-12) and a single Clostridia ASV (family Lachnospiraceae, unknown genus). It is possible that these organisms may associate with differential decay rates. Natural deaths were enriched for class 4C0d-2 (order MLE1-12) and ASVs in the classes Bacilli (family Lactobacillaceae, *Lactobacillus zeae*), Gammaproteobacteria (family Enterobacteriaceae, unknown genus), and Saprospirae (family Chitinophagaceae, genus *Sediminibacterium*). Among victims of accidental death, a single Bacilli ASV (order Lactobacillales, unknown family) and Gammaproteobacteria (family Enterobacteriaceae, unknown genus) were enriched. Homicide victims did not exhibit enrichment of any bacterial taxa. Currently, none of these signals was a significant predictor of manner of death.

#### Thanatomicrobiota, Reproductive Organs, 16S rRNA



### H163 The Postmortem Clostridium Effect: A Thanatomicrobiome Investigation of Cadaver Brain

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**Learning Overview:** After attending this presentation, attendees will have learned how to use the Postmortem Clostridium Effect as a biomarker for forensic microbiology in cadaver brains obtained from criminal casework with a postmortem interval of up to 11 days.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the role of *Clostridium* on the decomposition of human brains from actual criminal cases for forensic science applications.

What happens to the more than 100 trillion bacterial cells and 10 million human cells after a person dies has yet to be determined. It is known that brain cells generally die in as little as three to seven minutes after death. Revealing the diversity of microbial communities located in the human brain using bioinformatics and machine-learning tools have the potential to be a useful biomarker in forensic microbiology. The brain is identified as an intermediate-decomposing body site in the progression of the natural order of decomposition. Human thanatomicrobiome studies have established that putrefactive bacteria have been found in abundance within internal organs of decaying bodies. Of the various types of putrefactive bacteria found in internal body sites, obligate anaerobes of *Clostridium* spp. have been found in highest abundance. According to previous thanatomicrobiome studies, *Clostridium* spp. predominated at both short (4h) and long (up to 10 days) postmortem intervals.

The current study surveyed the postmortem microbiomes of brain tissues from 11 corpses with varying times of death ranging from 29 hours to 11 days. The brains of individuals were collected during autopsy using sterile equipment. Metadata for each cadaver was collected: age, sex, time of death, and cause of death from Official Daily Crime Logs. Total DNA was extracted using the phenol-chloroform method. *Clostridium* genes were amplified and sequenced using primers Clos58-F and Clos530a-R. The influences of case characteristics on microbiome composition, such as beta diversity, were investigated. Following standard demultiplexing and quality filtering using the Quantitative Insights into Microbial Ecology pipeline (QIIME™) and USEARCH global alignment algorithm, *Clostridium* spp. were identified. The output was analyzed using an internally developed Python program that assigned taxonomic information to each sequence. The results demonstrated that age was a significant predictor of the number of observed taxonomic units in brain samples. Furthermore, *Clostridium* and *Escherichia* were the most dominant observed bacterial genera in the study. To date, the results represent the first pilot study to perform *Clostridium* sequencing on postmortem brain tissue from criminal cases and confirm the Postmortem Clostridium Effect in decaying cerebral tissues.

#### PCE (Postmortem Clostridium Effect), Cadaver Brain, *Clostridium* Sequencing



## H164 Postmortem Cardiac-Magnetic Resonance (PMCMR) Protocol in Sudden Cardiac Deaths (SCDs)

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**Learning Overview:** After attending this presentation, attendees will better understand the advantages and limitations of postmortem imaging techniques in the study of SCDs. A protocol for an adequate image acquisition and its preliminary results will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by pointing out the importance of PMCMR and its ability to detect pathological myocardial alterations. It will be demonstrated that PMCMR is useful in the study of SCDs, showing a good correlation with autopsy results.

SCD is defined as abrupt and unexpected death that occurs due to cardiac causes, with sudden loss of consciousness within 1 hour from the onset of symptoms when death is witnessed, and within 24 hours of last being seen alive and well when it is unwitnessed. Its main characteristics are related to the non-traumatic genesis and the immediate precipitation of events. Sudden deaths account for 15%–20% of forensic cases. The most frequent causes of SCD are represented by Ischemic Heart Disease (IHD) secondary to Coronary Artery Disease (CAD), followed by primary cardiomyopathies (10%–15%; most frequently hypertrophic cardiomyopathy and arrhythmogenic right ventricular dysplasia), valvular heart disease (5%), and coronary artery anomalies (e.g., anomalies of origin, myocardial bridging). In 5% of cases, no structural cardiac alterations can be documented, but ion channel gene alterations (i.e., channelopathies) can be detected. However, in a minority of cases (5%–20%), the heart appears macroscopically and microscopically normal and no diagnosis can be established (*mors sine materia*).

In order to reduce the cases of death *sine materia*, it is necessary to adopt a correct and rigorous autopsy procedure, collecting and examining the clinical-anamnestic and circumstantial data. Also, Postmortem Magnetic Resonance (PMMR)—in addition to autopsy and classic gross/histological/immunohistochemical investigations—has proven useful in better detecting diagnostic possibilities, especially in the case of SCD.<sup>1</sup> PMCMR has been successfully applied as an ancillary exam to identify myocardial pathological findings, allowing visualization of thin transverse sections of myocardial tissue, which are not possible with routine autopsy and gross examination.<sup>2</sup>

Despite the fact that PMCMR has shown high sensitivity and specificity in the identification of heart diseases, it is currently performed only in a few select cases rather than routinely. The main reasons for its limited application seem to be related to the time of execution and the complexity of the MR technology. Moreover, most of the studies in literature describe PMCMR conducted prior to autopsy with the heart *in situ*. However, examining hearts *ex situ* shows considerable advantages, including overcoming logistical problems of body transport, time constraints, and interference with routine clinical activity. As an adjunct, fixation of the heart in formalin, as required by the autopsy protocol for SCDs, allows for preservation of the organ at room temperature, preserving from postmortem alterations and allowing for PMCMR examination a second time after the autopsy.<sup>3</sup>

Presented is a PMCMR study protocol of the entire formalin-fixed heart *ex situ* using high-resolution 2D-Steady-State Free Precession (SSFP)/3D-SSFP sequences, and T1-weighted (T1w), T2-weighted (T2w), and Proton Density (PD) Fast Spin Echo (FSE) sequences to obtain information on cardiac morphology, to identify ventricular anomalies (e.g., intramyocardial coronary bridging, aneurysms, symmetric or asymmetric hypertrophy of the ventricular wall, papillary muscle abnormalities, valvular anomalies, etc.) and to obtain quantitative parameters (i.e., Left Ventricular/Right Ventricular [LV/RV] mass, ventricular mass, atrial dimensions, aortic and pulmonary root diameters).

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### Sudden Cardiac Death, Postmortem Imaging, PMCMR



### H165 Radiodense Bullet Wipe (RBW) Around Skeletal Entrance Gunshot Wounds: The Frequency of Detection and Evaluation of Decedent, Wound, and Ammunition Characteristics

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**Learning Overview:** After attending this presentation, attendees will appreciate the prevalence of RBW around skeletal entrance gunshot wounds and the possible significance of bullet caliber, presence of jacket, and range of fire.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by documenting information that may be helpful for the postmortem evaluation of skeletal gunshot wounds.

“Bullet wipe” is the material deposited by a bullet on any surface with which it comes into contact after it is fired. Bullet wipe may contain debris from the gun barrel, including particles of primer and metal fragments from previously fired bullets. X-ray analysis is a non-destructive method by which traces of metallic elements can be visually detected. The analysis of bony defects for RBW is an important technique for assisting in the determination of the presence or absence of perforating gunshot wounds, especially in fragmented, skeletonized remains.

The aim of this current study was to determine the frequency of RBW around entrance firearms injuries that perforated bone. This study prospectively analyzed entrance gunshot wounds for RBW over a three-year period using digital X-ray analysis (KaVo NOMAD™ Pro with DEXIS™ software). The primary rate limiting step was the ability to maneuver the skeletal element for radiography. A total of 59 cases were able to be successfully radiographed. The autopsy reports from these cases were retrospectively reviewed to determine the frequency of RBW by biologic sex, reported ancestry, age-at-death, location of wound, manner of death, range of fire, bullet caliber, and presence of bullet jacket. Data were analyzed by Fisher’s exact test or Chi-square test (GraphPad Prism 8.1.2) with significance levels accepted at  $p < 0.05$ .

RBW was present in 66% ( $n=39$  of 59) of examined cases. Decedent biological characteristics did not significantly alter RBW, including biologic sex ( $p=0.75$ ), reported ancestry ( $p=0.49$ ), and age-at-death ( $p=0.43$ ) when examined by ten-year ranges. Additionally, the location of the bony entrance gunshot wound did not significantly affect RBW detection, including the frontal bone, right parietal bone, left parietal bone, right temporal bone, occipital bone, maxillae (hard palate bones), sternum, and mandible. RBW was not detected from the left temporal bone ( $n=1$ ) nor the rib ( $n=1$ ).

Manner of death did not significantly affect RBW detection, as RBW was found in 67% of homicides ( $n=18$ ) and 72% of suicides ( $n=21$ ). No RBW was detected from cases where the manner of death was classified as accident ( $n=2$ ) or undetermined ( $n=1$ ). Range of fire did not significantly affect RBW detection, as RBW was recovered from 65% of contact range wounds ( $n=22$ ), 69% of intermediate range wounds ( $n=9$ ), and 67% of indeterminate range wounds ( $n=8$ ).

When information was available regarding handgun cartridge caliber ( $n=49$ ), RBW was recovered from 100% of cases involving .22 caliber cartridges ( $n=3$ ), 50% of cases involving .25 caliber cartridges ( $n=1$ ), 64% of cases involving .380 caliber cartridges ( $n=7$ ), 56% of cases involving 9mm caliber cartridges ( $n=9$ ), 75% of cases involving .40 caliber cartridges ( $n=9$ ), 50% of cases involving .357 caliber cartridges ( $n=1$ ), and 33% of cases involving .45 caliber cartridges ( $n=1$ ). In 42 cases, the handgun cartridge was jacketed and RBW was detected in 67% of these cases ( $n=28$ ). All cases involving non-jacketed rounds ( $n=5$ ) showed RBW ( $p=0.30$ ).

Per research, this study is the first to report the frequency of RBW detection from skeletal entrance gunshot wounds.

#### Radiodense Bullet Wipe, Skeletal Gunshot Wound, Postmortem Radiology



### H166 Comparing Sinus Fluid Density in Drowning Versus Non-Drowning Victims Using Postmortem Computed Tomography (PMCT)

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**Learning Overview:** After attending this presentation, attendees will understand the implications of measuring the density of sinus fluid in drowning versus non-drowning victims using PMCT.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility of comparing sinus fluid density between drowning versus non-drowning victims and between fresh water versus ocean water drowning victims.

Drowning is usually a diagnosis of exclusion but there are characteristic autopsy findings described in the literature, including: presence of fluid in the paranasal sinuses and airways, pulmonary edema, water in the stomach, and mastoid air cell hemorrhage.<sup>1</sup> The content and characteristics of the fluid in the various sinuses are typically not analyzed when determining the cause of death. In PMCT, this anatomic area is easily visualized and the fluid character can be described in Hounsfield Units (HU). Prior studies have evaluated sinus fluid with PMCT. Christe et al. identified the amount and distribution of sinus fluid of 10 drowning cases compared to 20 non-drowning control cases.<sup>2</sup> Kawasumi et al. evaluated sinus fluid density and volume to differentiate salt water versus fresh water drownings.<sup>3</sup> A total of 93 cases (22 saltwater and 71 freshwater) were investigated, and it was found that saltwater drowning had a significantly higher fluid density than fresh water drowning.<sup>2</sup> In both studies, the sinus fluid density in HU was not compared quantitatively between drowning and non-drowning groups.

The goal of this study was to compare the HU of fluid in the sinuses of drowning versus non-drowning victims. PMCT scans from August 2016 to December 2018 were reviewed, and 68 total cases in which fluid was present in the sinuses were examined. Thirty-four drowning deaths were selected for the study group and 34 non-drowning deaths were selected for the control group. The controls included deaths from natural disease, accidents, and homicides. Head trauma was excluded from the controls as blood could be present in the sinuses. An equivalent number of males and females were selected for the drowning (26M, 8F) and control (27M, 7F) groups. The mean ages were 37.0 years (drowning) and 36.6 years (control). Radiologist consultants were blinded to cases and selected the appropriate Region Of Interest (ROI) and measured the average HU of fluid in the sinuses. The mean HU of drownings (25.0936) was lower than the mean HU of controls (30.6895), with a difference of 5.5959 ( $p=0.018$ ). The HU in drownings were compared by location (ocean [11] vs fresh water [23]). The mean HU of ocean (31.0636) was higher than the mean HU of fresh water (22.2384) with a difference of 8.8252 ( $p=0.0082$ ).

There was a difference in the mean HU of the drowning vs. control group, as well as in the ocean vs. fresh water group. While the presence of fluid is not a specific finding, the lower mean HU value in drowning deaths vs. controls represents a lower fluid density, consistent with a more watery fluid. Comparing the mean HU by location shows promising results and may have potential applications such as determining where a victim had drowned or if the body had been moved postmortem.

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#### Drowning, Sinus Fluid, Postmortem CT Scan



### H167 Heat-Induced Changes in Charred Human Remains

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**Learning Overview:** After attending this presentation, attendees will better understand how heat induces changes on human charred bodies and the possibility of distinguishing peri-mortem and postmortem bone fractures, taphonomic modification, and heat-induced bone changes by macroscopic observation and Postmortem Computed Tomography (PMCT).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing results of an observed case (January 2019) of charred human remains in an area (prolonged exposure of the human body to fire) that is very challenging to distinguish peri-mortem and postmortem fractures and by interpreting taphonomic modification and heat-induced bone changes.

Burned human remains in crime scenes are typically difficult to identify, recover, and manage.<sup>1</sup> All of the burned material at the scene, including body tissues, is often modified to a similar appearance. In particular, even though bone is certainly the most resistant to high-temperature exposure, at temperatures above 700°C, it will undergo complete combustion of the organic substances with incineration and recrystallization of the inorganic matter; this phenomenon is termed “calcination.” A charred or calcinated bone is known to be more fragile. The added peri-mortem or postmortem fracture lesion, fragmentation, and bone loss resulting from recovery techniques adds to the difficult task of autopsy and laboratory analysis of burned human remains. This is especially problematic for bone trauma analysis, as its most immediate goal is to distinguish peri-mortem trauma from postmortem alterations and shape lesions from heat-related traumatic lesions. With regard to burning-related changes such as shrinkage, the literature reports that low temperatures (i.e., less than 800°C) of minimal duration produce minimal shrinkage.<sup>2</sup> The characteristics of severely heat-damaged areas of tissue are usually obvious and well-known in forensic practice, but fractures may be challenging to interpret. In cases involving extreme exposure to fire, burned skeletal elements typically exhibit severe fragmentation and fracturing, limiting interpretation and distinction of peri-mortem and postmortem trauma.

In this study, a criminal case occurring on January 2019 and classified as Glassman and Crow level 4/5 male human remains is presented.<sup>3</sup> Color changes, fracturing pattern, bending, shortening, and cracking of all recovered bones by both visual examination and PMCT were analyzed. PMCT allowed for better recognition of cortical thinning and loss of matter, smoothing of the sharp edges, assessment of various alterations of bone marrow density associated with the presence of air, and recognition of thumbnail fractures as an exclusive sign of the burning of bones with soft tissues.<sup>4</sup> Preliminary statistical testing found a significant relationship (p-value <0.005) between the supposed temperature of combustion of analyzed bones within the Shipman color scale and the Hounsfield Units (HU) of bones on PMCT scan. Taphonomic modifications of the human remains were verified after four months and under controlled conditions before burial and are here illustrated.

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#### Charred Body, Heat Fractures, Incineration



### H168 Fatal Obstructive Asphyxia: Trans-Pulmonary Density Gradient Characteristic as a Relevant Identifier in Postmortem Computed Tomography (PMCT)

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**Learning Overview:** After attending this presentation, attendees will understand the key findings that may help identify cases of fatal obstructive asphyxia, such as smothering or manual strangulation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining how the forensic identification of fatal obstructive asphyxia may be of relevance when occurring early in the management of a given case. Hence, knowledge of relevant findings and their differential diagnoses may be of significance.

**Background:** The initial qualitative impression had been that obstructive asphyxia typically appeared to correlate with absence or reduced postmortem hypostasis as well as increased pulmonary radiotranslucency. Categories initially included within obstructive asphyxia were fatal aspiration of gastric contents into lungs, fatally blocked trachea or bronchi due to foreign body, fatal ligature strangulation, fatal manual strangulation, and smothering.

**Method:** To investigate the effects of obstructive asphyxia on the Postmortem Computed Tomography (PMCT) appearance of lungs, this study matched 24 cases of obstructive asphyxia with age-sex matched controls. Among the bodies that were examined routinely, there were a number of instances where there had been findings suggestive of some form of obstructive asphyxia: at the death scene, or when documenting the body's position in relation to the death scene, when examining the body. Routinely, such cases are subsequently admitted to this institute for PMCT and medicolegal autopsy upon which further findings emerge that then are evaluated in conjunction with the previous information.

Case selection was performed retrospectively by sampling all cases of 2014 through 2017 using criteria as detailed below. Only cases with full body PMCT, autopsy, and with a full final report were considered. Cases with fatal hypothermia, fatal blood loss, detectable injury of the lungs, or putrefaction with notable gas in soft tissues, vessels, or organs were excluded from the study.

This study manually placed density profile vectors, with a start at positionally dependent locations of each lung to derive PMCT densities for multivariate statistics. For each lung, such a vector was obtained, resulting in two vectors for each case.

As fatal gastric content aspiration cases did not significantly differ from the controls, they were re-grouped into the control group for further testing. The density vectors thus contained pulmonary densities starting from the positionally lowest to the opposite location of each lung.

**Results:** Quantitatively, the obstructive asphyxia case group featured reduced or absent postmortem hypostasis in the lungs and an overall lower density. Linear multivariate statistics (general linear models, linear discriminant analysis) performed relatively poorly, whereas "shallow" neural networks yielded significantly better discrimination. With disjunct training and test sets, neural networks achieved Correct Recognition Rates (CRR) of obstructive asphyxia cases of more than 95%.

**Conclusions:** Within a more narrowly defined collective of obstructive asphyxia, pulmonary PMCT data appears to contain sufficient information to reach a relatively high level of discrimination. This questions the value of an exclusively death scene-focused decision, making for further investigations and may justify a wider use of PMCT. Conversely, obstructive asphyxia remains difficult to diagnose conclusively based on PMCT alone.

#### Fatal Obstructive Asphyxia, Postmortem CT, Forensic Pathology



### H169 Using Postmortem Computed Tomography (PMCT) and Drug Screens to Triage Drug-Related Fatalities

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**Learning Overview:** The goal of this presentation is to present a novel method to triage drug-related death examinations using PMCT and drug screening.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing an alternative approach to managing the increasing numbers of drug-related deaths in a medical examiner's office without compromising accuracy.

**Background:** In the current context of rising numbers of drug-related fatalities and an overall shortage of forensic pathologists, the current National Association of Medical Examiners (NAME) recommendation that all drug-related fatalities receive a full autopsy may not be the most efficient use of increasingly limited resources. The New Mexico Office of the Medical Investigator (OMI) has devised a new triaging system using a combination of PMCT and drug screens to help alleviate some of the workload while still providing quality care.

**Methods:** Under the new guidelines, drug-related fatalities fall into one of five categories.

**Scenario 1:** The first category pertains to decedents 40 year old and younger, with no medical history, a scene suggestive of drug use, and a negative PMCT. If all of these criteria are met, the case is then triaged based on the results of a Urine Drug Screen (UDS), with a positive screen resulting in a conversion to external examination and a negative screen resulting in an autopsy.

**Scenario 2:** This category includes decedents with significant natural disease, regardless of age, or those that would meet the criteria for an external examination based on age or significant PMCT findings, but who also have some indicators for drug involvement based on history or scene findings. These cases may still receive an external examination with PMCT to document natural disease. A UDS is used in these cases to guide toxicology testing: a positive screen resulting in testing compatible with the positive screen and a negative screen resulting in no additional testing.

**Scenario 3:** The third category addresses decedents with lethal natural disease (i.e., hemopericardium) seen on PMCT or autopsy. The examination type depends on the modality by which the lethal natural disease is discovered. If found on PMCT, an external examination is appropriate. If found at autopsy, the exam type does not change. A UDS is used to guide toxicology testing (i.e., rule out drug involvement).

**Scenario 4:** The fourth category includes decedents with chronic ethanol abuse and significant natural sequelae of such on PMCT, but with some concern for acute ethanol toxicity. These cases may receive an external examination with confirmatory toxicology testing.

**Scenario 5:** The last category relates to all other drug-related deaths that do not fit into the first four categories (i.e., suicidal overdoses). At this time, these cases still receive a full autopsy.

**Results:** Within the period of May 2019 to July 2019, 150 cases were identified as meeting the criteria for one of the first four categories. These cases were triaged according to the above guidelines. During this time period, confirmatory toxicology testing was performed on all cases, regardless of drug screen results. Of those 150 cases, 124 (82%) were converted to external examinations with PMCT. A majority of these cases, 60%, were in Scenario 2, while 34% were in Scenario 1, and the remainder consists of Scenario 3 and 4. Drug screens were performed on 75% of the cases. For those cases with both a UDS and confirmatory toxicologic testing, sensitivity by drug category ranged from 75% to 100%, and specificity ranged from 77% to 100%. To date, of the cases that have been finalized, the UDS was in agreement with cause of death in all but one case. In this instance, the UDS was positive for known medications at therapeutic concentrations, which were determined not to have contributed to death.

**Discussion:** The conversion of 124 of 150 cases to external examinations resulted in an autopsy equivalent decrease from 150 to 57 (when using four external exams per autopsy equivalent). This is not only a significant reduction in workload per pathologist, helping maintain NAME guidelines for numbers of autopsies performed per year, but also reduced technologist labor and morgue resources. Additionally, in nearly 11% of cases with UDS, the drug screens were negative with negative confirmatory testing. In the future, these cases would not have needed confirmatory testing, reducing the cost of toxicology testing overall. The combination of PMCT and drug screens allows for a novel triaging system in which a full autopsy is not necessary for all drug-related deaths, decreasing workload while still maintaining accurate determination of cause and manner of death.

#### Postmortem Computed Tomography, Drug Screen, Autopsy





### H170 The Diagnostic Accuracy of Unexplained Intracranial Hemorrhage as an Indicator of Abusive Head Trauma in the Context of a Coagulopathy

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**Learning Overview:** The goal of this presentation is to present an analysis of the accuracy of the diagnosis of abusive head trauma when it is based solely on findings of intracranial and retinal hemorrhage and in the absence of any collateral evidence of abuse in a pre-mobile infant with a bleeding disorder.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility of a risk-based (i.e., epidemiologic) analysis when the cause of death determination in an infant is entirely dependent on a pathological finding with an unknown positive predictive value for intentional abuse and, thus, an unknown false positive (i.e., error) rate.

The case involved a 4-month-old male infant with no personal or family history of abuse, who was discovered pale, unresponsive, and not breathing by his father, around three days after he had been found to have a bump on the back of his head. Computed Tomography (CT) scan revealed an acute or chronic subdural hematoma and cerebral edema, with no skull fracture. By the time he had died five days later, the child had developed retinal hemorrhage. During the five-day hospitalization, it was found that the child had Factor VII deficiency, and months-later testing of his father revealed the same condition.

An autopsy conducted two days later noted a chronic subdural and subarachnoid hemorrhage, cerebral edema, optic nerve sheath hematoma, and retina hemorrhage. The cause of death was given as “blunt force injuries of the head,” despite an absence of any external sign of trauma. A child abuse pediatrician deemed the finding of both retinal hemorrhage and unilateral shin bruising (from intraosseous catheter) to be consistent with abuse.

Based solely on the fact that he was the caregiver who discovered the child in a non-responsive state, the father was charged with first degree reckless homicide. When confronted with the evidence of Factor VII deficiency, the pediatrician opined that this was secondary to, rather than a result of, the intracranial hemorrhage, noting that extensive personal experience with such injuries was the basis for the conclusion. The pediatrician also claimed that the injuries in the deceased infant, specifically the intracranial hemorrhage and retinal hemorrhage, are only possible due to physical abuse (i.e., shaking with or without impact) or a high-speed traffic crash or fall from at least a two-story height.

A forensic epidemiologic analysis of national hospital data was undertaken to assess the reliability of the claims made by both pediatrician and pathologist.

Data were abstracted from the Kids’ Inpatient Database (KID) and the National Inpatient Sample (NIS). The analysis included all children aged 1 year and under who were admitted to the hospital with intracranial and retinal hemorrhage from 2000 to 2014, inclusive. There were an estimated 9,493 children who met these criteria, approximately 633 infants per year. Coagulopathy was present in 406 (4.2%) of the hospitalized infants, or around 27 per year.

Of the 9,493 hospitalized infants, there were 5,014 (52.8%) who had no other diagnosed injuries that would be consistent with abuse (fractures, burns, internal injuries, etc.), or around 334 per year. Abuse was diagnosed or designated as a cause in 70.9% of the cases where there was no other diagnosed injury. Among the children who did not have a diagnosis of abuse, in the majority for which there was a cause specified, the injury resulted from a low-energy event, such as a ground-level fall or a fall from furniture or a bed.

The analysis revealed the fact that among pre-mobile children who are hospitalized for intracranial and retinal hemorrhage, both with and without coagulopathy, the injury did NOT result from abuse in at least one out of every five cases. Thus, the assertion by the pediatric specialist that, in the absence of a history of a high-speed traffic crash, etc., the injury must have resulted from abuse, was demonstrably false. The conclusion by the pathologist that the injury resulted from “blunt force trauma” was also rejected as factually baseless.

After being presented with the above evidence, in combination with the familial evidence of a coagulopathy, the state dismissed the homicide charge against the father.

#### Abusive Head Trauma, Retinal Hemorrhage, Factor VII Deficiency



### H171 Pediatric Accidental Deaths in Cook County, Illinois

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the incidence and circumstances surrounding pediatric accidental deaths in a busy, urban medical examiner's office during the past five years.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of pediatric accidental deaths in Chicago, IL, which may be useful in determining risk factors and prevention strategies for the pediatric population.

Accidental injuries are among the most frequent causes of death in children. According to the Centers for Disease Control and Prevention, unintentional injuries represent the fifth-leading cause of death in children <1 year of age and the first cause of death in children between the ages of 1–10 years.

A search of the database of the Cook County Medical Examiner's Office over the past five years (August 2014–July 2019) identified 131 accidental deaths that occurred in subjects between 0 and 10 years old. This represented 15.3% of all deaths occurring in the same period among children of the same age range (131 out of 852 cases).

The mean age was 2 years, and the median age was 1 year. The youngest (two infants) were five days old. The age distribution was as follows: <1 year old (58 subjects), 1 year old (16), 2 years old (13), 3 years old (7), 4 years old (6), 5 years old (13), 6 years old (5), 7 years old (2), 8 years old (2), 9 years old (7), and 10 years old (2). There was a male predominance, with 75 (57%) of the subjects being males and 56 (43%) females. By race, 66 (50%) of the subjects were African American, 58 (45%) were Caucasian, and 7 (5%) were other. Over the period analyzed, the peak incidences occurred in 2015 (35 cases) and 2018 (31 cases).

The cohort was divided into infants (less than 1 year of age) and children (1-10 years of age). The most common cause of accidental death in infants was asphyxia due to an unsafe sleeping environment (45 cases out of 58), followed by motor vehicle collisions, house fires, and drowning. In subjects over 1 year old, motor vehicle collisions, house fires, and drowning represented the vast majority of deaths (53 out of 73), followed by a number of events related to the size of the children and their cognitive and motor development. In particular, deaths associated with the ingestion of solid foods (choking on food bolus) and the capacity to walk autonomously (falls from height, entanglements in window shade cords, etc.) were observed in this age range.

Accidental injury in the pediatric population is frequent, and often a preventable cause of mortality. Prevention strategies rely on a correct evaluation of the causes of death and injury. Forensic pathology can help to identify risk factors and ways to reduce childhood mortality from injuries. This study has the intent to offer information for future intervention plans and a basis for future research.

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### Pediatric, Accidental Deaths, Unintentional Injury



### H172 Histologic Findings of the Pancreas in Infant Deaths: A Review of Cases at the State of Maryland Office of the Chief Medical Examiner (MD OCME)

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of some histological findings that may be seen in cases of infant asphyxia and/or Sudden Unexpected Death in Infancy (SUDI).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by alerting attendees to potential histologic findings in infant deaths attributed to asphyxia and/or SUDI.

SUDI is used to classify those deaths in children 12 months of age or younger with non-specific autopsy findings following a complete forensic autopsy that includes toxicology and radiology, review of medical history, and thorough scene investigation.<sup>1</sup> Most recently, the term SUDI has been used in situations involving infants found in unsafe sleep environments, such as prone positioning, bed sharing, and sleeping on surfaces not designed for infants (plush bedding, large pillows, couches, etc.). Confounding the diagnosis of SUDI, in cases of unsafe sleep environments, is that the risk of suffocation or asphyxia cannot be ruled out as the cause of death. In addition, “gentle” homicides, in particular smothering, can also have similar non-specific findings as SUDI and may be missed, which is why the majority of these cases should be ruled as undetermined.<sup>1</sup>

In a high-volume office such as the MD OCME, a majority of infant cases are ultimately ruled as SUDI, although in situations with strong supporting circumstances, such as a witnessed overlay or wedging, cases may be ruled as asphyxia. During routine review of infant autopsy slides from several autopsies, some sections of pancreas revealed inflammatory infiltrates that seemed independent of any systemic infectious process. Much is included in the literature about examining the histology of major organs, such as the heart and lungs, in cases of sudden infant deaths, but the pancreas is not typically included as a critical organ to sample. This raised the question of how often can such findings be seen in the pancreas on routine histology of infant cases? This led to the primary goal of this study, which was to review the histology of previous infant deaths in order to identify any possible trends. Another objective of the study was to see if there was a correlation between pancreatic infiltrates and the cause of death (i.e., asphyxia versus SUDI).

In order to recognize if pancreatic inflammatory infiltrates are a significant finding in infant deaths, a search of the MD OCME database was performed to identify all cases of infant deaths, ages 0 to 6 months, for the five years between 2013 and 2018. Pancreatic sections from a random selection of cases signed out as either “SUDI” or “Asphyxia” were included in the review. Excluded from the study were causes of death involving blunt force trauma, firearm injuries, and/or other natural disease processes, not inclusive of asphyxia. Per research, only one published article focused on the histopathologic findings in the pancreas in infantile asphyxia.<sup>2</sup> It is hoped that the results of this retrospective review will stress the importance of continually sampling those organs deemed “less important” in the context of sudden infant deaths and to potentially present findings that may force further investigation into these deaths.

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#### Pancreas, Infant, Deaths



### H173 Retinal (RH) and Optic Nerve Sheath Hemorrhage (ONSH), Papilledema, and Spinal Cord Nerve Root/Ganglia Hemorrhage Associated With a Cerebral Cavernous Malformation

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**Learning Overview:** After attending this presentation, attendees will learn that extensive RHs, ONSH, and papilledema can occur in young children who have increased intracranial pressure, and spinal cord nerve root/ganglia hemorrhages are not specific for hyperflexion-hyperextension injury in young children.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reinforcing the necessity for routine postmortem ocular and spinal cord examinations of children with fatal intracranial hemorrhage, cerebral edema, and elevated intracranial pressure.

RH and ONSH in young children have been considered specific for inflicted traumatic brain injury. Current studies suggest that non-traumatic, markedly elevated Intracranial Pressure (ICP) rarely causes RHs, and when it does, the RHs are few in number and peripapillary. Dorsal root ganglial hemorrhage has been attributed to hyperflexion-hyperextension neck injury (shaken baby syndrome) in infants and young children. A case of a young child with papilledema, bilateral RHs, ONSHs, and spinal nerve root/ganglia hemorrhages associated with non-traumatic intracranial hemorrhage arising from a ruptured left temporo-parietal cavernous malformation is presented. The clinical, autopsy, and investigative findings of this case refute the purported specificity of RHs, ONSHs, and spinal dorsal root ganglial hemorrhages in young children.

**Case Report:** A 5-year-old boy with no significant medical history was found unresponsive at home. At the local hospital, cranial Computed Tomography (CT) revealed a large intraparenchymal hemorrhage. The previous day, he had been diagnosed with Streptococcal pharyngitis and sent home with antibiotics following complaints of fever and headache. On arrival at the tertiary medical center's Pediatric Intensive Care Unit, he had dilated pupils and a Glasgow coma scale of 6. A repeat cranial CT revealed extensive intraparenchymal hemorrhage centered in the left temporal lobe measuring to 6.1cm, extensive surrounding edema and mass effect resulting in 11mm rightward midline shift and crowding of suprasellar cistern concerning for uncus herniation, multifocal subarachnoid hemorrhage along the left convexity, and intraventricular extension of hemorrhage into lateral ventricles with developing hydrocephalus. His family offered no reports of recent trauma. His initial ICP was 67mmHg and remained elevated (40–50mmHg) despite external ventricular drain placement and mannitol treatment. Due to the extent of his neurological injury and high ICP that was refractory to treatment, his parents decided to withdraw care; he was pronounced dead 26 hours after admission. No clinical fundal examination was recorded in the medical record.

Postmortem indirect ophthalmoscopy revealed bilateral papilledema (left > right), numerous splinter, flame-shaped, and dot retinal hemorrhages over the posterior poles and extending past the equators. His autopsy was significant for a 7 x 5 x 4cm intraparenchymal hemorrhage of the left temporo-parietal cortex with associated cerebral edema, left uncus herniation, and cerebellar tonsillar herniation. The intraparenchymal hemorrhage was from a ruptured, thrombosed, necrotic cavernous malformation. Diffuse spinal cord nerve root and ganglial hemorrhages involved the cervical, thoracic, and lumbar regions. The RHs were multilayered and the bilateral ONSH was intradural, subdural, and subarachnoid.

This case describes severe hemorrhagic retinopathy, papilledema, ONSH, and diffuse spinal cord nerve root/ganglia hemorrhages associated with a fatal spontaneous, non-traumatic, intracranial hemorrhage arising from a ruptured cavernous malformation of the left temporo-parietal lobe. The reported ocular and spinal cord findings have been considered diagnostically specific for abusive head trauma (shaken baby syndrome); however, this case illustrates the need for caution in attributing these findings as diagnostically specific for abusive head trauma. This case emphasizes the importance of consistent and thorough postmortem ocular and spinal cord examination in young children with intracranial hemorrhage, cerebral edema, and elevated intracranial pressure.

#### Retinal Hemorrhages, Papilledema, Increased Intracranial Pressure



## H174 Recognizing Congenital Syphilis: The Consequences of the Return of an Epidemic

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**Learning Overview:** After attending this presentation, attendees will better understand the epidemiology and findings of congenital syphilis in the neonate.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the potential impact of the current syphilis epidemic on the forensic community and highlight the characteristics of congenital syphilis that can be found at the time of autopsy so an appropriate workup and diagnosis can be made.

**Introduction:** Syphilis, despite being an ancient infection, remains a significant global health concern with newborn syphilis cases reaching a 20-year high in 2017.<sup>1</sup> This study describes an accidental death of a premature infant with concurrent congenital syphilis and amphetamine toxicity. With infection rates rising and neonates of women with poor prenatal care and substance abuse at particular risk, the importance of medical examiner and coroner offices being able to recognize congenital syphilis is pertinent.

**Case Description:** The decedent was a less-than-24-hour-old prematurely born female infant at approximately 34-35 weeks gestation. The mother was unaware of the pregnancy until presenting to an emergency department with vaginal discharge and pain. During the admission, a congenital rash was noted, initiating an infectious workup and a *Treponema pallidum* antibody enzyme immunoassay that was positive. The case fell under medical examiner jurisdiction due to a positive maternal urine drug screen at the hospital. At autopsy, anomalous facies and a diffuse skin rash were present. Congenital pneumonia, acute pancreatitis, and inflammatory skin changes were present in histologic sections, consistent with underlying congenital syphilis. Toxicology studies revealed levels of amphetamine in the blood.

**Discussion:** Syphilis is caused by *Treponema pallidum*, a motile spiral-shaped Gram-negative bacterium, with a steady increase in congenital infections since 2012. Congenital syphilis cases within the United States has more than doubled since 2013, with 918 new cases reported in 2017, including 13 infant deaths, coinciding with increases in rates of primary and secondary syphilis in women; 70% of the cases occurred in Florida, California, Arizona, Texas, and Louisiana.<sup>1</sup> An important risk factor for congenital syphilis is underutilization of prenatal care, with substance use as one of many obstacles to accessing care.<sup>2-4</sup> In congenital syphilis, there is widespread dissemination of spirochetes to almost all organs, with most frequent and severe involvement of the bones, liver, pancreas, intestine, kidney, and spleen. While pathologic changes in the fetus can be seen as early as 15 weeks gestation, two-thirds of live-born cases can be fully asymptomatic, depending on the timing of infection *in utero*. Clinical manifestations of early congenital syphilis appear by three months of age in untreated infants, with most common manifestations including hepatosplenomegaly, mucocutaneous involvement (skin maculopapular eruption, desquamation, and crusting), and skeletal anomalies (periostitis, cortical demineralization, and osteochondritis). Manifestations of late congenital syphilis, arbitrarily defined by clinical manifestations with onset after two years of age, include anomalous facies, interstitial keratitis, Hutchinson teeth, palatal perforation, and anterior bowing of the shins. Congenital syphilis should be suspected in all infants born to women with positive non-treponemal and treponemal tests for syphilis and in infants and children with compatible clinical findings. The diagnosis of syphilis requires direct identification of the bacteria or by positive serologic findings.<sup>5</sup> This case is an example of the impact of the growing syphilis epidemic on medical examiner and coroner's offices and a review of congenital syphilis features.

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### Syphilis, Congenital, Rash



### H175 Cervical Vertebral En Bloc Examination in Pediatric Deaths: The New York City Experience (2011–2019)

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**Learning Overview:** The goal of this presentation is to inform attendees regarding neuropathologic features of spinal nerve root hemorrhages and associations with other features of trauma, hypoxia-ischemia, or sepsis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating that cervical vertebral en bloc dissection is a proposed means of detecting traumatic spinal nerve root injuries, hypothesized to lead to cardiorespiratory arrest in children suffering shaking and/or impact. Whether Cervical Spinal Nerve Root Hemorrhages (CSNRH) can occur because of hypoxia-ischemia, sepsis, or other non-traumatic etiologies is difficult to study prospectively, as the dissection technique is non-standard, invasive, and thus pursued only in deaths “suspicious” for inflicted trauma. The goal was to evaluate retrospectively the range of findings in all “neck block” specimens since the subject was first published in 2011 and whether traumatic and non-traumatic cases could be discerned.

**Methods:** All pediatric cases from 2011 to the present in which this technique was done were reviewed. The dates of birth, presentation, and death, as well as the findings from forensic autopsy, neuropathology, and microbiology, were analyzed along with final manner of death. Given the exploratory nature of this study, statistical methods were not employed.

**Results:** Over eight years, 43 infants and toddlers (age at death, 1 day to 3.25 years; median 5.3 months) were subject to this protocol because of suspicious or unknown circumstances. Twenty (46.5%) had bilateral, multilevel CSNRH, defined as hemorrhage *within* the spinal nerve root and/or dorsal root ganglion; specifically, 11 had *bilateral* involvement at C3, 4, and 5 (responsible for diaphragmatic innervation). Seventeen of these 20, as well as 12 other cases without bilateral multilevel CSNRH (i.e., 74.4% of the cohort), had other evidence of trauma, including scalp impact, skull/rib/other bone fractures, and/or blunt force abdominal injuries, and were ruled as homicides. Co-occurrence of bilateral multilevel CSNRH, Subdural Hemorrhage (SDH), and bilateral Retinal/Optic Nerve Hemorrhages (RH/ONH) was documented in 19 of the 20. Importantly, four of these cases had *no evidence* of head impact (scalp lesions, skull fractures). In addition to the 20 with bilateral multilevel CSNRH, another 4 had unilateral and/or single level involvement; among these 24, 18 (75%) had Hypoxic-Ischemic Encephalopathy (HIE), associated with resuscitation, life support, and survival >1 day; the remaining 6 of this subset died within one day, without time to develop HIE. Of note, four individuals dying within one day and, therefore, without HIE (3 with bilateral multilevel and one with single level SNRH) also had Amyloid Precursor Protein (APP) immunohistochemical patterns of traumatic axonal injury, further suggestive of torsional force injury to the brain. Among all individuals with HIE ( $N=29$ ), 15 had bilateral multilevel CSNRH; 7 had very limited bleeding around nerve roots (including one infant with confirmed viral sepsis with bacterial superinfection, certified as natural), or foci of endoneurial hemorrhage in a single cervical level. The remaining seven had no hemorrhage whatsoever in or around nerve roots and did not have SDH or RH/ONH (including one infant with suspected sepsis, certified as natural). APP immunostains in 9 of 29 cases (31.0%) with HIE showed mixed patterns of single beaded axons in sites typical for traumatic axonal injury, as well as bundled axon staining typical of ischemia. Of note, however, was the fact that eight of these nine also showed bilateral multiple CSNRH, SDH, and RH/ONH, and were ruled as homicides on the totality of the evidence.

**Conclusions:** Over an eight-year period, this agency adopted “neck block” dissection in pediatric cases with unclear or suspicious circumstances at time of autopsy. Like all other data considered in death certification, neck block findings of bilateral multilevel CSNRH do not “stand alone,” but rather provide additional support for trauma, as they tend to cluster in the majority of cases with objective signs, such as scalp impact, skull/other bone fractures, and abdominal injuries. Particularly remarkable is that, in the absence of evidence of any head impact, bilateral multilevel CSNRH is associated with both SDH and RH/ONH and with markers of diffuse axonal injury, thereby supporting the role of fatal shaking as a mechanism of death. Nearly half of cases with HIE, and the small number of deaths due to sepsis, do *not* show this pattern of nerve root hemorrhage, arguing against non-traumatic pathogenetic mechanisms underlying this phenomenon. Finally, because of the apparent value of this technique to medical examiners in their work-up of suspicious deaths in infants and toddlers, this agency intends to continue its use.

### Abusive Head Trauma, Cervical Nerve Root Hemorrhage, Child Abuse



### H176 Maternal Death Investigation in the Setting of Rising Maternal Mortality in the United States: Trends in Maryland From 2003 to 2019

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**Learning Overview:** The goal of this presentation is to highlight maternal death investigation in the forensic setting, to emphasize the characteristics of this at-risk population, as well as the utility of proper documentation for the benefit of public health.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing awareness to the public health issue that is the increasing United States Maternal Mortality Rate (MMR) by reinforcing the role of medicolegal death investigators, coroners, and forensic pathologists when it comes to investigation of deaths in women of childbearing age.

MMR has been steadily increasing in the United States since 1987, reaching 31.2 maternal deaths per 100,000 live births as documented in 2016, despite the United Nations Millennium Development Goal for a 75% decrease in maternal mortality from 1990–2015. Focusing on “preventable” causes of maternal mortality—those which may have been averted by one or more changes in the health care system related to clinical care, facility management, public health infrastructure, and/or patient factors—is an important step in addressing this national public health issue. Involvement of medical examiner and coroner offices is vital in the initiative to better understand the underlying cause of maternal deaths and explain changes in MMRs.

From January 2003 with preliminary data up to the current date, 345 maternal deaths were reported to the Office of the Chief Medical Examiner (OCME) in Maryland. While relatively uncommon in the forensic setting, these cases highlight characteristics of this at-risk population, as well as the utility of proper documentation for the benefit of public health.

The Maryland OCME records from 2003 onward were searched for reported cases in which the “pregnant in the last year” box was checked—an addition Maryland made to all death certificates in 2001. These deaths were then subclassified as follows: cardiovascular conditions (32.2%), intoxication/substance abuse (16.5%), non-cardiovascular medical conditions (13.3%), homicide (12.5%), injury/accident (12.5%), infection (3.8%), hemorrhage (3.5%), cerebrovascular (2.6%), and suicide (2.9%). One case was classified as undetermined (0.2%).

Pregnancy-related deaths are those which occur within 365 days of pregnancy and are related to or aggravated by pregnancy or its management. Of the 345 deaths reported to the Maryland OCME over a 17-year period, 42% were identified as pregnancy-related (excluding homicide, intoxication/substance abuse, injury/accident, and infectious causes unrelated to pregnancy). Pulmonary embolism was the leading cause of cardiovascular deaths (29%). Beginning in 2013, a shift in the trend was noted in that deaths related to intoxication/substance abuse surpassed those related to cardiovascular disease (26.1% intoxication vs. 20.3% cardiovascular). However, pulmonary embolism (8%) remained the leading cause of pregnancy-related cardiovascular death.

Between two five-year periods (2007–2011 and 2012–2016), the United States MMR increased by 37.2% (reaching 28.4 deaths per 100,000 live births), while the Maryland rate decreased by 7.6% (23 deaths per 100,000 live births). While trends for more recent years in Maryland have improved, there is still work to be done to achieve the national and global goals for maternal mortality rates (target 11.4 deaths per 100,000 live births). In our population, cardiovascular disease remains a leading cause of pregnancy-related deaths, with pulmonary embolism being the most prevalent cause of death in the peri- and postpartum period. Nationally, cardiovascular conditions were responsible for greater than one-third of pregnancy-related deaths from 2011–2015. Globally, obstetrical hemorrhage followed by hypertensive disorders account for the majority. Forensic pathologists and medicolegal investigators need to be aware of these deaths and at-risk populations so as not to misdiagnose and/or misclassify these deaths. Encouraging other providers to report these deaths to medical examiner/coroner offices, as well as proper and accurate documentation on death certificates by forensic pathologists, will help measure the true magnitude of the problem, identify at-risk populations, and allow health departments to focus protective efforts on women who need it the most.

#### Maternal Mortality, Pregnancy-Related Deaths, Reporting Maternal Deaths

### H177 A Histological Study of Persistent Pulmonary Hypertension of the Newborn (PPHN): A Five-Year Retrospective Analysis of a Fatal Cause in Neonates

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**Learning Overview:** After attending this presentation, attendees will be aware of the specific histological features of the lung in PPHN and understand their prognostic significance.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of a disease process that may present as unexplained cardiopulmonary arrest in neonates during and after birth hospitalization, giving rise to medical malpractice litigations and lawsuits.

PPHN is a serious condition that occurs in about 1.8–2 per 1,000 newborns (both term and preterm) in the neonatal intensive care unit, and may significantly complicate the clinical course of approximately 10% of neonates with respiratory insufficiency.<sup>1-3</sup> It is associated with an increased risk of an adverse outcome, including neurologic impairment in 15%–25% of neonates with PPHN who survive after hospital discharge.<sup>4</sup> PPHN is defined as a failure to achieve or sustain the physiological decrease in pulmonary vascular resistance at birth associated with the persistence of the typical *in utero* right-to-left shunt. PPHN may eventually lead to life-threatening circulation failure and is one of the main causes of unexpected death of neonates, with a mortality rate of about 10%–20% of the affected patients.<sup>5</sup> PPHN is often associated with structural or functional disorders of the lungs or systemic diseases, such as diaphragmatic hernia, meconium aspiration, pneumonia, and placental insufficiency.

Clinically, PPHN is characterized by tachypnoea, severe cyanosis, acidosis, and rapidly increasing hypoxemia. PPHN diagnosis is confirmed by echocardiographic evidence of increased pressures in the pulmonary artery and right heart chambers in the absence of evidence of congenital heart disease; however, conclusive diagnosis of PPHN-related death currently relies on autopsy examination. Typically, the clinical manifestations are noticeable leading to the antemortem diagnosis of PPHN. Nevertheless, PPHN arising suddenly and early after birth is usually fatal, despite evidence-based treatment. PPHN remains a challenge in therapeutic management because the complex etiopathogenesis is yet to be fully explained. Moreover, prenatal assessment of PPHN is very difficult and not always possible.

The present study comprised a five-year retrospective analysis of data from neonatal autopsies collected in two hospitals in Northwest Italy (i.e., “Ospedale Ginecologico Sant’Anna,” Torino, and “Ospedale Pediatrico G. Gaslini,” Genova). It entailed thorough identification and analysis of histologic findings in PPHN cases. The aims of the study were to enable better prediction of lung function outcomes in affected neonates and potentially help clarify cases of medical malpractice litigation. From January 2014 to December 2018, the hospital-selected units in Torino and in Genova performed 1,312 and 456 autopsies, respectively, of which 86 and 111 were on neonates. There were 39 autopsies that involved neonates affected by PPHN in life (i.e., 18 in Torino and 21 in Genova). Thus, the average incidence of PPHN as a cause of death among all neonatal autopsies was 19.79% (i.e., 20.93% in Torino and 18.92% in Genova).

Paraffin-embedded tissue sections from the lungs of all 39 autopsy cases were cut in 2- $\mu$ m slices and stained with different histologic and immunohistochemical stains. Specific histologic parameters (i.e., degree of lung development, morphological features of bronchioles, alveolar veins and capillaries, interstitial septae structure, presence of arteriolar layers thickening, hyaline membranes, intralveolar histocytes, interstitial or endoalveolar inflammation, and therapy-induced pulmonary alteration) were reevaluated on light microscopy.

In accordance with the literature, this study showed PPHN is a severe syndrome frequently encountered in pediatric hospitals. Moreover, it is often responsible for the sudden death of neonates during hospitalization after birth. This presentation offers attendees an insight into the importance of an in-depth knowledge of histologic criteria in PPHN that may enhance appreciation of the severity of the disease, guide better management, and help predict pulmonary response. This is fundamental to significantly reduce the rate of neonatal mortality and the associated medicolegal implications.

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#### Histological Features, Persistent Pulmonary Hypertension, Newborn





### H178 The Utility of Postmortem Vitreous Beta-Hydroxybutyrate (BHB) Testing for Distinguishing Sudden From Prolonged Deaths and for Diagnosing Ketoacidosis

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**Learning Overview:** After attending this presentation, attendees will better understand the utility of postmortem vitreous BHB levels in prolonged and sudden natural and non-natural deaths and will be provided with values that may be used to establish a diagnosis of ketoacidosis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing postmortem vitreous BHB threshold levels for the diagnosis of ketoacidosis as a cause of death.

Elevated BHB has been used in the diagnosis of Diabetic (DKA) and Alcoholic (AKA) ketoacidosis, with BHB values above 2.5mmol/L in blood often utilized as a threshold. Recently, vitreous BHB has been established as a reliable alternative to blood. It has been shown that vitreous BHB levels above 6mmol/L (with glucose levels above 200mg/dl) are strongly associated with DKA. Slightly elevated levels of BHB have been noted in other stressful states, including infection and hypothermia. The objective of this study was to determine whether elevated postmortem vitreous BHB can be used to predict stressful types of deaths and to establish a vitreous BHB threshold for the diagnosis of ketoacidosis as a cause of death.

A retrospective cross-sectional analysis of vitreous BHB was performed during a two-year study period. Extracted data included history of diabetes mellitus and alcoholism, vitreous glucose, electrolytes and BHB levels, and cause of death, in addition to demographic information. Cases were excluded if the decedent was less than 18 years of age, where collection of samples was not possible, or where the cause of death was not determined. Analysis of BHB was performed using enzymatic quantification by beta-hydroxybutyrate dehydrogenase. Cases were sorted into six categories of death: (1) sudden non-natural death (ST); (2) sudden natural death (SN); (3) prolonged non-natural death (PT); (4) prolonged natural death (PN), (5) DKA; and (6) AKA. Statistical comparisons were made between sudden and prolonged natural and non-natural deaths using the Student's *t*-test. Comparison between alcoholic and diabetic ketoacidosis cases used the non-parametric Mann-Whitney method. Analyses were conducted using Microsoft® Excel® and Minitab® 16.

Nine hundred sixty-seven cases met the inclusion criteria. The mean age was 51.7 years (median=53 years) with a range from 18–97 years. Six hundred twenty-six cases were male and 341 were female. One hundred twenty-two cases had a known history of diabetes and 121 a history of alcoholism (17 cases overlapped). The mean BHB for all cases was 1.67mmol/L (17.4 mg/dl), and ranged from 0.11–18.02mmol/L. For the categories of death, the number of DKA, AKA, PN, PT, SN, and ST deaths were 21, 5, 155, 258, 275, and 253, respectively. Their mean vitreous BHBs were as follows: 11.04mmol/L (DKA), 8.88mmol/L (AKA), 1.56mmol/L (PN), 1.55mmol/L (PT), 1.26mmol/L (SN), and 1.38mmol/L (ST). There was a statistically significant difference between the mean BHBs of the prolonged and sudden natural deaths ( $t=3.54$ ;  $p$ -value <0.001). A significant difference was also seen between the mean BHBs of the prolonged and sudden non-natural deaths ( $t=2.92$ ;  $p$ -value=0.004).

In addition, this study sought to identify a threshold for vitreous BHB levels where ketoacidosis was determined to be the cause of death. BHB levels between 2.5 and 5mmol/L produced specificities in the high nineties and sensitivities above ninety.

While these results show a statistically significant difference of vitreous BHB levels between prolonged and sudden natural and non-natural death, the values do not appear to hold clinical significance. This study has, however, shown that vitreous BHB levels in the range of 2.5–5mmol/L can be set as a threshold for determining ketoacidotic deaths, in line with results previously published for blood BHB.

#### **Beta-Hydroxybutyrate, Vitreous, Ketoacidosis**



### H179 3D Rendering of the Human Body: A Proposal of an Operative Protocol for the Application of Photogrammetry in the Autopsy Room

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**Learning Overview:** After attending this presentation, attendees will have an overview of the benefits of photogrammetry applied to forensic pathology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing an operative protocol that allows accurate and simple data acquisition that can be used during postmortem examinations.

Forensic pathology has evolved in the modern era, thanks to new techniques aimed at improving the bodies' evaluations. The external and internal examinations are still critical to gather information, representing the gold standard for the investigation of the cause and manner of death.

The external examination represents the first approach to the body, allowing the forensic pathologist to gather information about the state of the body and the presence of tissue lesions. Unfortunately, due to the decompositional process, if an injury is missed during the external examination, it can be hard to identify even on subsequent re-examination of the body. At the same time, one of the most relevant issues with the autopsy is that it is an invasive, destructive, and non-repeatable analysis, so that the findings often cannot be accurately re-assessed.

To overcome these problems, taking photographs of the body before starting the postmortem examination is a crucial part of the medicolegal death investigation process. Photographs must be accurate and detailed since they are taken mainly for legal purposes and are intended to be shown in court. However, even photographs do not allow for a detailed review of an injury or an organ. Their main limitations stand in the 2D rendering of the human body, which has three dimensions, and in their non-repeatability.

Currently, there is an increased interest in "forensic photogrammetry." Photogrammetry is a tool that allows the making of measurements from photographs. It represents an accurate and low-cost method that can be easily implemented in the routine medicolegal death examination.

This study proposes a step-by-step protocol based on a 3D acquisition of the body and applying photogrammetry during the external examination. This technique makes it possible to obtain a 3D graphic reconstruction of the body based on single photographs. Photos must be taken following specific criteria to allow an accurate and reliable photogrammetric rendering. The 3D information is obtained through the acquisition of several partially overlapping photos. After that, a dedicated software recognizes points and areas with similar features and builds a 3D mesh of the body. Although any camera can be used to take photographs of the body (e.g., smart phone), the use of a good-quality camera is highly recommended to obtain the best results. At the end of the image processing, the software will display a 3D rendering of the body, showing the morphology, the texture, and allowing for measurement. The body will be shown as it is observed during the external examination, overcoming potential issues with bad quality single photos or missed lesions. Moreover, it is possible to use photogrammetry to obtain 3D documentation of internal organs or any other element of forensic interest that may be encountered during the postmortem examination.

The advantages of photogrammetry lie in its low cost, simple application, and accurate results. The 3D rendering admits the body rotation on a 360° axis, allowing for a better interpretation and presentation of the features of forensic interest, which can be helpful in court. This is particularly relevant in cases of homicides: in such situations, displaying the 3D images of the deceased to the court will make it easy to show the location and the features of any lesions, allowing a straightforward demonstration of the condition of the body before the autopsy. The main limitation of this technique is that it is time-consuming. A full photographic report requires taking pictures of the anterior and posterior aspect of the body, with an estimated time ranging from 20 to 40 minutes. Moreover, a good source of illumination is needed, and a clean background is preferable to avoid software misinterpretations.

It is believed that photogrammetry is a reliable and useful tool in forensic practice. The application of this protocol allows a simple and accurate 3D documentation of the body during the different phases of the autopsy.

#### Photogrammetry, Autopsy, Protocol



### H180 Electrolytes, Glucose, and Lactate in Postmortem Blood (BL), Vitreous Humor (VH), and Synovial Fluid (SF): A Comprehensive Study

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**Learning Overview:** After attending this presentation, attendees will better understand the comparative and correlation study of sodium, potassium, chloride, magnesium, glucose, and lactate levels in postmortem BL, VH, and SF of the same subject as well as the correlation study between the constituent concentrations in the fluids and various factors, including gender, age, body height, body weight, body mass index, and Postmortem Interval (PMI), the essential basic knowledge that can fill the current gap in intelligence about using the biochemical substance levels in the fluids to solve any forensic issues in daily practice or further research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as essential knowledge of postmortem investigation of sodium, potassium, chloride, magnesium, glucose, and lactate levels in BL, VH, and SF for forensic applications that have been challenging the forensic community worldwide.

In forensics, sodium, potassium, chloride, magnesium, glucose, and lactate levels in certain human body fluids have been demonstrated to change over time since death. However, there is still controversy regarding their changes in the fluids, and no studies have explored their levels in postmortem BL, VH, and SF simultaneously. Thus, such changes were investigated in the fluids of 52 forensic autopsy cases with an intact body, no metabolic disorders, and no functional or structural abnormality of the eyes or knees, who were autopsied within 15 hours after death. This study also compared and correlated the values from the fluids and determined the effects of gender, age, body height, body weight, Body Mass Index (BMI), and PMI on the parameter concentrations.

Study results showed that BL potassium, magnesium, glucose, and lactate values were significantly higher than the VH and SF values. The opposite pattern was seen for VH sodium levels and VH and SF chloride levels ( $p < 0.001$ ), where SF sodium values were equivalent to the BL values. VH and SF sodium, potassium, chloride, and lactate values had significant relationships with the BL values ( $p < 0.05$ ), but not for magnesium and glucose values. BL potassium and magnesium levels were significantly higher in men than in women, but the opposite pattern was seen with BL chloride levels ( $p < 0.05$ ). Only SF magnesium levels had a negative correlation with the body height of the decedents ( $p < 0.05$ ). This study also found positive linear correlations between BL, VH, and SF potassium concentrations and the PMI as well as between VH and SF lactate concentrations and the PMI, but negative correlations with the PMI for BL sodium, BL chloride, and VH glucose concentrations ( $p < 0.05$ ). Among these relationships, VH potassium has the strongest correlation with PMI ( $r = 0.67$ ,  $R^2 = 0.45$ ), followed by BL potassium ( $r = 0.56$ ,  $R^2 = 0.32$ ) and SF potassium ( $r = 0.49$ ,  $R^2 = 0.24$ ) with respective high prediction index. The parameters that change over the PMI are unreliable indicators of antemortem levels unless the samples are obtained very shortly after death. In addition, the low rates of change of certain parameters with low predictability of PMI make them of very limited use in estimating the PMI. Other parameters served better as biomarkers of underlying conditions or causes of death. The variable effects of the state of the decedents, such as gender and body height, should also be taken into account whenever considering the use of certain biochemical substances for application to specific issues in forensic practice.

#### Postmortem Biochemistry, Postmortem Chemistry, Thanatochemistry

### H181 The Usefulness of a Hand-Held Blood Glucose and Ketone Monitoring Device as a Postmortem Indicator of Diabetic Ketoacidosis (DKA)

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**Learning Overview:** The goal of this presentation is to describe the preliminary results of a study aimed at assessing the performance of a point-of-care blood glucose and ketone monitoring device in measuring glucose and  $\beta$ -hydroxybutyrate concentrations in postmortem vitreous and blood samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the potential usefulness of hand-held point-of-care devices in screening cases of DKA in a postmortem setting.

**Background:** DKA is a potentially life-threatening complication of type 1 diabetes mellitus. Glucose and ketone bodies such as  $\beta$ -Hydroxybutyrate (BHB) can be measured postmortem in Vitreous Humor (VH) to assess antemortem hyperglycemia and ketoacidosis. However, such analyses may be technically challenging and expensive as they involve sophisticated biochemical methods. As Point-Of-Care (POC) Blood Glucose and Ketone Monitoring Devices (BGMD) are known to provide rapid and accurate results in living patients, they may be used as a reliable and cost-effective approach to screen potential DKA cases at the autopsy, as suggested by prior research.<sup>1</sup> This preliminary study aimed to assess the performance of such a device in measuring glucose and BHB concentrations in postmortem VH and blood samples.

**Materials and Methods:** VH and blood samples were collected from patients known or suspected to be diabetics at the autopsy. VH and blood specimens were collected in dry and lithium heparin tubes, respectively. Glucose and BHB concentrations in VH were measured twice in both eyes, before and after centrifugation (5,000g, 5min), with a commercially-available FreeStyle® Precision Neo BGMD, according to the manufacturer's instructions. BHB levels were also measured in blood using the same device. The results were compared to those obtained with validated biochemical methods. For glucose, reference levels were measured from perchloric acid precipitated VH samples with enzymatic hexokinase/glucose-6-phosphate dehydrogenase method. Values  $\geq 7$ mmol/L were considered to be elevated. BHB concentrations in VH and blood were measured with enzymatic Hydroxybutyrate Dehydrogenase (HBDH) method. Values  $\geq 3$ mmol/L were considered to indicate ketoacidosis. The study was approved by the Ethics Committee of the University Hospital of Montpellier.

**Results:** Forty-five subjects (33 males, 12 females) were included, with a mean age of  $56.1 \pm 13.4$ . Twenty-six subjects were known to be diabetic patients. The postmortem interval was  $3.4 \pm 2.1$  days. No statistically significant difference was shown between both eyes for glucose and BHB levels, and this study observed a high reproducibility of measurements in each eye. Glucose and BHB levels measured before VH centrifugation were not significantly different from those after centrifugation. There was a strong correlation between VH glucose concentrations measured with the BGMD and the validated method ( $R^2=0.74$ ,  $\rho=0.72$ ,  $p<0.0001$ ). The mean values obtained with the BGMD were significantly higher to those obtained with the validated method (7.4mmol/L vs. 5.2mmol/L,  $p<0.05$ ). The sensitivity of the BGMD to detect cases with elevated VH glucose levels was 1.0 and the specificity 0.97 when the threshold value was set to 10mmol/L. No correlation was found between BHB values measured with the BGMD and the validated method, whether in VH or in blood. The sensitivity of the BGMD to detect elevated BHB levels in VH was 1.0 but its specificity 0.38 with a threshold value of 2.5 mmol/L, while they were both 1.0 to detect high BHB concentrations in blood with a threshold value of 3.0mmol/L.

**Conclusion:** This preliminary study confirms that BGMD may be useful in identifying potential DKA cases in postmortem settings. The results obtained so far suggest that glucose can be reliably investigated in VH, regardless of the eye and without any prior centrifugation, whereas BHB analysis should rather be considered in blood. Further analysis on additional samples is underway for confirming these preliminary results and for better delineating appropriate threshold values.

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#### Point-of-Care Device, Diabetic Ketoacidosis, Postmortem Screening



## H182 Lung Weights in Carbon Monoxide (CO) -Related Fatalities

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**Learning Overview:** After attending this presentation, attendees will be familiar with the mechanism of death in CO-related fatalities and also recognize the possible effects of CO on the weight of the lungs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by determining if a relationship exists between the presence of postmortem carboxyhemoglobin and the weight of the lungs at autopsy in CO-related fatalities.

CO is a colorless, odorless gas that is produced from the incomplete combustion of organic fuel.<sup>1</sup> It causes hypoxia by preferentially binding to the hemoglobin molecule in place of oxygen, producing carboxyhemoglobin.<sup>1</sup> Commonly recognized as a “silent killer,” CO is the most common lethal poisoning in the United States.<sup>2</sup> While suicides constitute the majority of CO-related fatalities, over 2,200 deaths resulted from unintentional CO poisoning from 2010 to 2015 in the United States.<sup>2,3</sup> Non-smokers generally have carboxyhemoglobin concentrations of less than 3% at baseline, depending upon the setting in which they live (e.g., urban versus rural).<sup>1</sup> However, smokers may have a slightly higher hemoglobin concentration, though usually less than 10%.<sup>4</sup> Therefore, carboxyhemoglobin can be detected in the blood of decedents who die under a wide variety of circumstances, from the 50-pack-a-year cigarette smoker with chronic obstructive pulmonary disease to the arson victim found dead in his home. Other than the “cherry-red lividity” and the bright red viscera often seen on examination, the autopsy findings in acute CO toxicity are not well characterized.<sup>2</sup> As it can act as a central nervous system and respiratory depressant, similar to opioids, which are frequently associated with pulmonary edema at autopsy, lung weights in CO-related fatalities were evaluated.

This study retrospectively reviewed cases of CO-related fatalities. A search was completed using an online database of deaths investigated by medical examiners and/or coroners from several counties in Michigan and Indiana from 2008 to 2019. This study restricted the search to include the following terms in any portion (a, b, c or d) of the immediate cause of death on the death certificate: *carbon monoxide, inhalation, exhaust, and combustion*. It was also restricted by type of examination, as this study required cases with a full autopsy in order to review the postmortem lung weights. In addition, cases were excluded if carboxyhemoglobin testing had not been completed.

Twenty-eight cases of CO-related fatalities were identified, not including those due to fire exposure. In a study by Molina and DiMaio, average normal lung weights were determined by evaluating postmortem lungs in sudden, traumatic deaths of adults.<sup>5,6</sup> On average, normal male right lungs weighed 445g and left lungs weighed 395g, while normal female right lungs weighed 340g and left lungs weighed 299g. Of the 28 CO-related deaths, 22 were male, ranging in age from 20 to 74 years, and 6 were female, ranging in age from 19 to 71 years. The right lung in the deceased males ranged in weight from 450g to 1,120g, while the left lung ranged in weight from 270g to 1,040g. This resulted in an average of 782g for the right lung and 710g for the left in males. The right lung in the deceased females ranged in weight from 350g to 660g, while the left lung ranged in weight from 370g to 640g. This resulted in an average of 536g for the right lung and 493g for the left in females. The average percentage of postmortem carboxyhemoglobin was 56.7 among all male and female CO-related deaths.

Compared to the aforementioned normal lung weights, the right and left lungs in male CO-related fatalities were both 1.8 times heavier and in female CO-related fatalities were both 1.6 times heavier. As this data continues to be evaluated, fire-related fatalities that include a component of CO inhalation will be examined for comparison.

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### Carbon Monoxide, Lung, Carboxyhemoglobin



### **H183 A Model for Estimated Heart Weight With Accounting for Estimated Cardiomegaly**

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**WITHDRAWN**

### H184 Unrecognized Anatomical Larynx Variants May Lead to More Than 70% of False Larynx Fractures

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**Learning Overview:** After attending this presentation, attendees will acquire a greater understanding of anatomical laryngeal variants, as well as their impact on the diagnosis of fractures of the hyoid bone and thyroid cartilages.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the pitfalls associated with anatomical variants in strangulation cases. Additionally, the estimation of the incidence of false laryngeal fractures based on the prevalence of these variants, already established for a Galician population (Northwest Spain), will also be of interest to the scientific community.

Misinterpretation of fractures of the hyoid bone and thyroid cartilage due to anatomical laryngeal variants are common because the variants are usually unknown or poorly recognized. This comes as no surprise considering that forensic textbooks and the forensic literature have failed to pay attention to these anatomical variants, namely with epidemiologic studies assessing their prevalence.<sup>1,2</sup>

After having identified in 207 consecutive autopsies the most frequent anatomical laryngeal variants (i.e., triticeal cartilage (52.7%), terminal segmentation of the thyroid horns (11.7%), ectopic superior thyroid horns (8%), lateral thyro-hyoid ossification (5.3%), and calcification of the stylohyoid ligament (1.4%)) this study devised a mock exercise to quantify the impact of these variants on the diagnosis of either thyroid or hyoid fractures.<sup>3</sup>

In order to achieve this objective, a case-by-case evaluation exercise was carried out by two experienced pathologists. Taking into account the particular characteristics and specific location of each variant, its potential misinterpretation was recorded. An estimation of the false positive rate was calculated considering the number of anatomical variants possibly misclassified as fractures to all samples in the study.

The results showed 71.5% potential errors (false positives) of the 207 cases studied.<sup>3</sup> Triticeal cartilages were the most important variant affecting the diagnosis of laryngeal fractures, with a rate of potential errors of 46.4%, followed by the terminal segmentation of the thyroid horns (7.3%), ectopic superior thyroid horns with 6.3% of possible errors, and the lateral thyrohyoid ossification (4.4%). In addition to the isolated variants, in 6.8% of the individuals, there were more than one anatomical variant with the potential to influence the diagnosis.<sup>3</sup> The most frequent association was the terminal segmentation of the thyroid horn with triticeal cartilage that may lead to 3.8% of false fracture diagnoses. It should be noted that only 28.5% of the cases would be totally exempt of any negative influence of anatomical variants.

These results demonstrate the necessity for forensic pathologists to be aware of laryngeal anatomical variants in order to avoid erroneous interpretation of autopsy findings. Apart from the increasing use of X-ray and computed tomography as ancillary techniques, the manual dissection of the neck, with observation and palpation of the fractures, remains the major tool for forensic pathologists worldwide. This reinforces the importance of these results and the need for a correct interpretation of the anatomical variants when identifying laryngeal fractures in strangulation.

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#### False Larynx Fractures, Larynx Anatomical Variants, Triticeal Cartilage



## II Demons and (Mis)Diagnosis: A Cultural Case Study of Sleep Paralysis

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**Learning Overview:** The goals of this presentation are to: (1) review the core symptoms and prevalence of Sleep Paralysis (SP) with hypnagogic hallucinations; (2) describe the impact of cultural context in the interpretation of SP symptoms; and (3) identify the potential errors in psychiatric diagnoses of patients with SP.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping attendees appreciate the varied manifestations of SP in different cultural settings, allowing for accurate diagnoses and referrals. Attendees will increase cultural competency, avoid erroneous diagnoses, and effectively consider SP in the differential diagnosis of hallucinations.

SP is a relatively widespread phenomenon in which individuals awaken and are unable to make voluntary muscle movements. This phenomenon is frequently accompanied by hypnagogic hallucinations, often of a threatening figure or presence. SP is considered a parasomnia, a sleeping disorder, if it recurs frequently and is distressing. SP has a lifetime prevalence rate of 7.2%, with students and psychiatric patients experiencing SP more frequently.<sup>1</sup> It often accompanies other sleeping disorders, such as narcolepsy.

Interpretation of SP, especially the hallucination of an evil being, varies between cultures and countries. The “hag” in Shakespeare’s *Romeo and Juliet* is considered to be a manifestation of SP, while in Brazilian tradition, there is the character of Pisadeira, an old woman who lurks on rooftops and crushes sleepers’ chests.<sup>2</sup> More recently, individuals interpret their hallucinations during SP as part of an alien abduction.<sup>3</sup> The cultural context of interpreting the symptoms of SP has a significant impact on the way individuals seek and receive treatment for SP; due to cultural stigmas, they may turn to confidential sources (such as herbalists and shamans) for help rather than seek treatment at medical institutions.<sup>4</sup>

Forensic evaluators routinely assess for the presence of psychotic symptoms but may not consider SP as an etiology. The differential diagnosis of hallucinations typically focuses on primary psychotic disorders, such as schizophrenia or substance-induced psychosis. *The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)* does not include SP in the differential diagnosis of psychotic disorders, but does include schizophrenia in the differential diagnoses of narcolepsy.<sup>5</sup> Accordingly, an evaluator’s index of suspicion for a sleep disorder may be low in the context of bizarre and threatening hallucinations. An evaluator may err in diagnosing a major psychotic disorder or attributing “atypical” symptoms to malingering instead of referring an evaluatee for a sleep study. Errors in diagnosis may expose an individual to unnecessary treatment or result in a damaging label of “malingerer.” This presentation illustrates the potential for error with a forensic case study of a man who interpreted vivid SP-related hallucinations as an evil demon spirit that threatened him. He interpreted the hallucination as a signal of his need to improve his religious practices. His family, who attended the same Baptist church, endorsed this interpretation. However, forensic evaluators concluded he was malingering and did not refer him for further treatment or evaluation.

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### Sleep Paralysis, Hallucination, Culture





### I2 A Mystic Religious Figure Who Became an Actress—From a Man of God to a Showgirl: A Particular Case of Circumvention of the Mentally Incapable Perpetrated Against a Couple, as Well as Crowds of People

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**Learning Overview:** After attending this presentation, attendees will more fully appreciate the importance of knowing how to recognize the various signs of circumvention of the mentally incapable and the need to take the necessary steps, both in its prevention and in response to it.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that circumvention of the mentally incapable comes in many forms, some obvious and others not so obvious. New ways to address this phenomenon must be formulated and put into practice.

In Italy the offence of circumvention of the mentally incapable is defined in art. 643 of the Penal Code as follows: “*whoever, to gain profit for himself or others, abuses the needs, passions or inexperience of a minor, or abuses the state of infirmity or psychic deficiency of any person, even not subject to interdiction or declared mentally incapable, and induces this person to accomplish an act that has any harmful judicial consequences on himself or others, is punished ...*”

Victims of such crimes are people who normally conduct a normal, well-adapted existence, but who may be affected by some fragility, unexpressed desires that have not been fulfilled, traumas, unresolved problems that have not been processed, or even psychopathologic traits, characterized by a tendency for dependency, low self-esteem, or a highly suggestible personality. An examination of the relationship between the perpetrator and victim in these types of crimes is essential during the related clinical and legal investigations.

**The Case:** In a rural area in southern Italy, where daily fears and hopes for the future seek an ear and reassurance against the background of the anguish of their daily life, Mr. B. and Mrs. D. came to this study’s attention, bound by a marriage that was over in all but name. They had been selected by a self-proclaimed seer and mystic as the “adepts and beneficiaries” of his “powers.”

The circumstances of “P. the mystic” are those of the last born of a large family living on a single salary, a minor and self-proclaimed seer, who over time built up a reputation for monthly apparitions of Our Lady, attracting crowds of the faithful and huge donations for fantastic projects aimed at saving the world. He came to know the above-mentioned couple and promised intercession with God to allow them to create a family and receive the blessing of children.

“P. the mystic” gained the trust of both, and love of both at different times, sometimes purely carnal, also in a homosexual relationship, and at others involving profound submission, obedience to making love to the person indicated, and total credulousness, as when instructed to believe in a pregnancy in the man. The financial (about two million euro) and emotional costs (two relationships outside marriage for each of them), presented as the means to happiness, mark an episode that is in its own way unique, exceptional, and terrible, as curious as it is unlikely, unhappy and distressing for the parties.

Manipulations and suggestions ensnared them. Data on reality were freely interpreted in a mystic-grandiose manner in a type of delirious trance. True events were afforded the same credibility as parables or prophecies. The absolute remission to the “divine will” appears more as the expectation of a Messianic destiny to which fears and responsibilities are continually subordinated than as a true, aware act of faith.

**Conclusions:** In this view of the world, often driven by a preponderant belief in magic that is seen as a form of religion, it is reasonable to suppose that the suggestions insinuated could have achieved the dual function of reassurance and credibility.<sup>1</sup> It is well known that religions in general, to respond to these needs and be presented as meaningful systems, are proposed as communication systems built on narratives, social forms, and actions.<sup>2,3</sup> In particular, by offering shared beliefs, stories, and meanings, religions contribute to confer order to social reality, proposing various forms of “communicative contract,”<sup>and</sup> how the destinies of such proposals accept them opens up ample scenarios offering different possibilities and positions.<sup>4,5</sup>

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#### Crime, Forensic Evaluation, Circumvention



### I3 Mental Health and Stress Relating to Crime Scene Investigators (CSIs): A Lack of Training, Support, and Resources for CSIs

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**Learning Overview:** The main goal of this presentation is to compare self-perceived stress between civilian CSIs and sworn officers, the impact on their mental health, as well as the available stress management tools. After attending this presentation, attendees will have a better understanding regarding the inconsistencies about reporting and recognition of occupational stress experienced by civilian CSIs and their sworn law enforcement counterparts. Significant differences apparently exist in the training, screening, coping strategies, and stress management between the two groups. There are also different standards in place to contend with exposure to traumatic events and related stress exposure and availability of resources for stress management for these professionals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community and the agencies employing CSIs by demonstrating the need for more awareness of the occupational stress regarding this group, more open mental health discussions, and research to address these concerns. This is especially relevant for civilian CSIs, where stress and mental health support is nearly non-existent.

The plan of this study was to use a 28-question anonymous survey that collected responses about the stress perceived and experienced on the job; the training and education regarding stress management and coping skills; and the awareness of resources, policies, and support once stress or trauma is experienced. Participants within this study included CSIs ( $N=12$ ) and sworn law enforcement officers ( $N=46$ ) whose duties included responding to scenes of death, violence, dangerous, or emotionally sensitive scenes.

The results indicated that both groups were statistically similar in reporting that their agencies or employers offer programs of peer support, assistance, or critical incident stress management, with 100% of respondents having acknowledged that the services existed. Another area of the study evaluated the perceived stress experienced by both groups during the course of their duties. The results demonstrated there was a two-fold difference in reporting stress for the CSIs compared to the sworn officers. The self-reported scaled responses were also statistically analyzed and showed twice as many CSIs having experienced vicarious stress as compared to sworn officers. The topic of training and education in stress management within the first year of hire demonstrated 75% of CSIs did not feel they received adequate training compared to 30% of sworn officers. The perception of similar resources for assistance once stress was encountered showed 75% of CSIs thought they did not receive assistance, compared to sworn officers (28%).

In conclusion, the survey responses from this study suggest CSIs feel they are experiencing stress and trauma at similar levels as sworn officers and that sworn officers appear to have been given more training and education to cope with the stress encountered.

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#### Crime Scene Investigation, Mental Health, Stress



## I4 A Rare Case of Psychotic Serial Killing by Poisoning

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**Learning Overview:** After attending this presentation, attendees will know the atypical case of an Italian serial poisoner and how the case has been analyzed in a forensic context.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of the rare possibility that cases of intoxication can result from criminal behavior and of the complexity of a forensic psychiatric assessment.

There is no recent international literature on serial poisoners and, historically, this criminal behavior has been described more commonly in females than in males.<sup>1,2</sup> This is the atypical case of a 27-year-old Italian man who killed three members of his family (grandfather, grandmother, and uncle) and poisoned four other members of his family by the hidden administration of thallium. The thallium was ordered from an Italian pharmaceutical company, bought directly and paid for in cash. In recent months, the man developed his plan to kill seven members of his family, not including his parents. The man was arrested by the police after a brief investigation. Upon detainment in jail, he talked to the psychiatrist about the reasons for his crimes: he had to punish them by death for their “idolatry,” which in his mind was their poor sense of decency. This consisted of behaviors such as wearing more comfortable clothing at home. He said that he tried to convince his family to change and adapt their behavior to “conform to the will of God,” but they did not listen to him, so he was forced to kill them. He also mentioned having heard voices that ordered him to punish his family members for their indecent behavior. He was finally voluntarily admitted to a psychiatric acute ward, treated with antipsychotic drugs, and discharged to prison after several days with a diagnosis of “Delusional Disorder.” He underwent to a brain Computed Tomography (CT) scan to exclude a significant organic disorder.

For the previous three years, he had been showing significant social withdrawal, loss of external contacts, and initiated a concentrated study of religious texts without any basis in real life or recognized religious authority. Based on these observations, the judge requested a psychiatric evaluation to assess, as allowed by the Italian criminal law, his capacity to stand trial, his mental state at the time of the murders, and the presence or absence of social dangerous. He was evaluated by four psychiatrists and tested by a psychologist. Two psychiatrists were requested by the prosecutor, one by the defendant’s advisor, plus there was the judge’s advisor. Thus three different forensic psychiatric assessments were produced: two assessments supported Not Guilty by Reason of Insanity (NGRI) and one identified only a diminished responsibility. During the evaluation, the defendant told his story again and remained inflexible with respect to his murderous intents, showing lack of empathy toward his victims and his parents. In this regard, he showed no remorse and said that he did not poison his father and mother only because he did not want to draw attention to him. The presence of a delusional disorder with a mystical-religious theme was clear and part of a complex psychotic disorder that brought him to consider himself a type of “avenger” of the will of God. The patient is currently hospitalized in a secure forensic unit.

**Conclusions:** This rare case of a serial poisoner provides an opportunity to examine how an offender’s ability to plan and execute an antisocial act with some apparent “lucidity” during the action should not exclude a possible diagnosis of schizophrenia. In such cases, the motive for murder is often revenge, triggered by persecutory delusions.

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### Serial Killing, Poisoning, Case Report



## I5 Big Data and Machine Learning: Changing the Risk Assessment Landscape

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**Learning Overview:** The goal of this presentation is to provide attendees with an introduction to the process of machine learning and the available models as well as an overview of the potential ways in which it can be implemented into forensic psychiatry practice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing understanding of machine learning and the benefits of incorporating machine learning into forensic psychiatry. In addition, attendees will gain insight into the application of machine learning in the risk assessment process and future directions for using this type of analysis.

The risk assessment field has seen little change since the introduction of actuarial and structured professional judgement tools in the 1990s. At the time of their introduction, these tools greatly improved risk assessment practice and outcome. However, for the past 10–15 years, other than tools being tweaked and new versions being released, there has been no notable advancement in the field. Information technology and data storage have greatly advanced research practice in a number of fields in recent years, and machine learning and the use of big data have the potential to change the risk assessment landscape.

Big data consists of a large amount of data, created at a high velocity and of a wide variety of types. Given how complex these datasets are, it is unpractical to use traditional statistics to analyze them. Moreover, statistical analyses identify risk factors at a group-level instead of an individual level and often do not deal well with the comorbidities and heterogeneity of psychiatric disorders. Machine learning analysis can be used to extract value from big data and transform it into applicable information. Machine learning is a field of artificial intelligence that includes analyzing current and historical trends to make predictions on how unseen cases will behave as well as identifying patterns of human behavior to detect trends. The primary aim is to allow the algorithms to learn automatically and adjust the actions accordingly, with minimal human intervention.

The use of traditional statistical models has often focused on group effects, which makes it hard to extrapolate what is found to a specific individual, and are often used in conjunction with theoretical frameworks in order to confirm prior assumptions. However, because machine learning is data-driven and looks at patterns underlying the data itself, it provides the opportunity to identify predictors and automatically adapt and customize them on an individual level and potentially display patterns that are very useful that were previously unexpected.

While still in its infancy, research utilizing machine learning and big data in psychiatry are showing great promise. Predictive models may help to develop tailor-made interventions for patients and a better allocation of resources from the health care system. The process of machine learning and the data-driven models will be highlighted, as well as their application in forensic settings, as these models have the potential to identify individuals at a higher risk for an outcome of interest and can aid in the prediction of violent behavior, criminality, and social rehabilitation.

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### Machine Learning, Big-Data Analytics, Forensic Psychiatry



### I6 The Electronic Hamilton Anatomy of Risk Management-Forensic Version (eHARM-FV): Launching the Fifth Generation of Risk Assessment

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**Learning Overview:** After attending this presentation, attendees will have been provided with an introduction to the eHARM-FV tool, the patient analytics function, and the role of the eHARM-FV in improving patient outcomes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by ensuring that attendees have acquired a better understanding of the eHARM-FV tool, the application of it in practice, and the potential benefits of using data analytics in forensic psychiatry.

The HARM-FV is a structured professional judgment tool that was developed in an effort to enhance the short-term risk assessment and risk management processes in forensic psychiatry. The HARM-FV combines static and dynamic risk factors to facilitate team discussions of risk and guide treatment planning, privilege requests, and risk management. By guiding through relevant risk factors, the HARM-FV facilitates a continuous process, eventually guiding the team to make an informed judgment regarding risk.

After nearly a decade of successful use and given the richness of the data captured by the HARM-FV and the potential for research advancement, the functionality of the HARM-FV was increased through the development of the innovative eHARM-FV. The eHARM-FV was developed in an effort to bridge the gap between psychiatry and technology by combining the risk assessment and risk management processes with built-in analytics to enhance and inform these processes. The use of technology to measure, monitor, and assess risk and change would have a significant impact for key stakeholders within psychiatry, including patients, care providers, and the community.

The eHARM-FV automatically generates individual , which allows users to easily track changes at an individual level. A patient's progress can be viewed on specific risk factors, aggressive incidents, and to track changes in clinical risk ratings over multiple time periods. These patient analytics using real-time data are accessible to the team at any point during or after a risk assessment and consequently allow users to target treatment and potentially enhance everyday clinical practice. Users can refer to the analytics during an assessment as a way to track decompensation or improvement and inform the assessment process. These analytics may also allow teams to better distinguish antecedents to specific incidents or behaviors, then use this information to inform future treatment or interventions. Thus, the eHARM-FV combines the risk assessment and risk management processes with built-in analytics to enhance and inform these processes in a way never before seen by forensic psychiatry.

The eHARM-FV has been successfully implemented in several psychiatric settings in Canada, with additional versions, translations, and training opportunities now available. Through this presentation, an overview of both the patient tool and patient analytics will be provided, and learning regarding the use of analytics in forensic psychiatry and the ways in which such a tool can enrich clinical practice in this setting will be discussed.

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#### Violence Risk Assessment, Data Analytics, eHARM



### I7 **Advancing Risk Assessment and Risk Management Using Analytics: The Electronic Hamilton Anatomy of Risk Management-Forensic Version (eHARM-FV)**

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**Learning Overview:** This presentation will provide a demonstration of the eHARM-FV aggregator and database functions as well as results from analyzing eHARM-FV-based data with machine learning.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by imparting knowledge about the eHARM aggregator and how eHARM-FV-based data can be analyzed using machine learning.

The eHARM-FV is the first risk assessment strategy to incorporate and use internal analytics and “big data” capabilities and thus introduces the fifth generation of risk assessment. With the capability of the eHARM-FV to store data, an additional tool, the aggregator, was created. The development of the aggregator tool added a significant degree of functionality that heightens the usefulness of the eHARM-FV. By loading multiple eHARM-FV patient files into the tool, the aggregator allows for large-scale analysis at the group level and within moments, trends can be viewed across diagnosis, risk factors, treatments, and interventions. For instance, users can easily identify which programs have the highest number of referrals, greatest involvement, longest waitlists, and least engagement to inform program planning and resource allocation. Moreover, this data may be cross-referenced with aggressive incidents or risk ratings to determine where a need exists for a given program or unit. Thus, the eHARM-FV database can inform risk management, research, service planning, and quality management.

In addition, the aggregator tool allows users to download imported data into a de-identified database that includes each existing eHARM-FV report for each patient selected. The database derived from the eHARM-FV is generated at the clinical interface, avoiding additional data collection or entry and the potential for errors, and increasing the ecological validity of studies that use the eHARM-FV-based data. This function of the aggregator presents a step toward machine learning and big data analysis within forensics as it creates a rich database consisting of an extensive variety of relevant variables at numerous time points, alongside accurate, longitudinal, and historical data about risk, treatment, and outcome variables.

Big data and analytics are rapidly changing health care and enabling a degree of measurement and quality improvement not previously seen. For a variety of reasons, including the limited number of quality indicators in mental health care, psychiatry has been late to the game. Forensic mental health has a few advantages in that there are recordable indicators within the behaviors that are monitored and measured. Use of technology to measure, monitor, and assess risk and change would have a significant impact for key stakeholders, including patients, care providers, and the community. Analytics offers an opportunity to increase our understanding of the forensic population, target effective programs and interventions, and direct more personalized care at the critical intersection of risk assessment and prediction—risk management. The eHARM-FV takes advantage of the capabilities afforded by big-data analytics to enhance the assessment, monitoring, and management of risk at the clinical interface, as well as the opportunity to use machine learning in forensic psychiatry. Through this presentation, the aggregator tool will be demonstrated and preliminary findings using machine learning to analyze eHARM-FV-based data will be discussed.

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**eHARM, Data Analytics, Forensic Psychiatry**



## I8 An Overview of Forensic Patients Within the Ontario Review Board (ORB) Patient Database

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**Learning Overview:** The goal of this presentation is to provide attendees with an overview of the Ontario forensic psychiatry system and with a representative profile of individuals who are under the jurisdiction of the ORB.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by expanding current knowledge and answering a number of key questions in regard to individuals in the forensic psychiatry system who have been found Unfit to Stand Trial (UST) and Not Criminally Responsible (NCR), as well as the implications for decision-making in forensic psychiatry.

The purpose of this study was to provide a representative profile and increase the understanding of forensic patients who are under the jurisdiction of the ORB. In Canada, those who are deemed UST or NCR on account of mental disorder are placed under the jurisdiction of a Review Board. There is a Review Board in each province, each of which assumes the role of determining the custodial status of the UST and NCR accused.

In Ontario, individuals found UST or NCR are placed under the jurisdiction of the ORB. Consequently, these individuals become the subject of annual review board hearings, in which hospital reports are prepared for board members to review; these include personal, family, psychiatric, and criminal history as well as treatment response, progress, and risk profiles. These reports are provided to assist the review board members in determining whether the accused should remain incarcerated or receive a conditional or absolute discharge. In making these decisions, the review board members are required to take into consideration the need to protect the public, the mental condition of the accused, and the reintegration of the accused into society.

The ORB, hospital staff, and the field of forensic psychiatry, in general, are responsible for the tremendously difficult task of identifying at-risk individuals, making decisions regarding one's ability to be tried for and held responsible for their criminal actions, and making decisions regarding an individual's ability to live safely in the community. It is imperative that institutions and tribunals are as informed as possible in making these decisions, which can only occur with sufficient empirical research and evidence.

At St. Joseph's Healthcare Hamilton, a total of 1,241 annual ORB hospital reports from the years of 2013–2015 were collected from ten forensic psychiatry facilities and coded to create the ORB Patient Database. The database includes male (86%) and female mentally ill offenders, all of whom are over the age of 18 years ( $M=42.54$ ,  $SD=13.33$ ). All individuals in the database were either found UST (3%), NCR (78%), permanently UST (5%), or previously UST and subsequently found NCR (13%) for a violent (70%), non-violent (20%), or sexual (10%) index offense. The primary objectives of this study were to use the ORB Patient Database to: (1) describe historical profiles of ORB patients, (2) identify trajectories, (3) examine index offense characteristics, (4) evaluate reported risk levels and assessments, and (5) psychiatric profiles.

In order to best inform and enhance the decision-making progress of the ORB, it is imperative to understand who the forensic psychiatry population is, what factors predict specific outcomes, and how to improve trajectories within the system. The current study will aid in expanding current knowledge and answering a number of key questions, including, but not limited to, those listed and informing forensic decision-making for years to come. Further results and implications from the current study will be discussed.

### Review Board, Forensic Psychiatry, Mental Disorder



## **I9 Is the Canadian Review Board Effective? An Evolving Forensic System Depending on Case Laws**

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**Learning Overview:** After attending this presentation, attendees will be aware of how the forensic psychiatry system functions in Canada. Attendees will know the power that the Review Board has to ensure community safety and allow psychiatric rehabilitation. Attendees will also be aware of how the Review Board and the Forensic Psychiatric programs interconnect to achieve these goals. Attendees will be aware of the case laws that have defined the roles of the Review Board and that have recently changed some of its functioning.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing a system that is based in Canada to an audience that will likely be international. As the Canadian Review Board is a system that is focused on psychiatric rehabilitation, this presentation shows the efficiency but also the difficulties that professionals in forensic mental health may encounter.

The Canadian Review Board system has comprehensive procedures to allow individuals who suffer from a mental disorder and have committed a crime for which they were found to be not criminally responsible based on a mental disorder or permanently unfit to stand trial to be rehabilitated. Even though the “paramount” criterion is community safety, the Review Board has to order dispositions that will permit the patients to attend programs that will benefit their rehabilitations, notably therapeutic interventions that will address their risk factors. Based on a federal legislation, the Review Boards and its applications are described in the Criminal Code of Canada. Each hearing held by the Review Board allows a thorough analysis of the patients' situations. Its members are part of a multidisciplinary panel (lawyers, clinicians, and public members), allowing for the acquisition of several view points on the situation to decide about the most appropriate dispositions, tailored to the clinical requirements for both the community and the patient.

Case laws have been instrumental in refining the legal requirements and prerogatives of the Review Board. This presentation will highlight the legal mechanisms under which the Review Board operates. It will also present how some of the most recent decisions of the Court of Appeal have changed some outcomes and how the evidence needs to be presented. This presentation will provide a description on how the Review Board system operates and stimulate a discussion with attendees that will likely offer some international perspectives.

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### **Criminal Code of Canada, Forensic Psychiatry, Risk Mitigation**





## I10 The Legalization of Cannabis in the Canadian Review Board System

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**Learning Overview:** The goal of this presentation is to discuss how legalization of cannabis may have an impact on conditions of discharge of individuals previously found not criminally responsible due to a mental disorder. Attendees should learn how a shifting landscape in terms of cannabis legalization and adoption of harm-reduction techniques may influence the discharge conditions of individuals in the forensic system.

**Impact on the Forensic Science Community:** This presentation will have an impact on the forensic science community by informing attendees about how legalization of previously illegal substances could lead to changing tolerance of substance use for individuals previously found not criminally responsible due to a mental disorder.

In the Canadian Review Board system, when a person is found not criminally responsible for a crime due to a psychiatric disorder, the Review Board may prohibit them from the use of any illegal substances if they are known to have abused substances in the past. Often the clause on an individual's disposition will dictate that the individual abstains from all illicit or illegal drugs. Up until recently, that clause encompassed cannabis use. In recent months, however, this prohibition has become more complicated, as cannabis is no longer considered an illegal substance in Canada.

As a result of this change, there has been a recent shift in how the Review Boards consider substance use prohibitions for individuals found not criminally responsible. One consequence of the legalization of cannabis has been individuals and their physicians seeking that prohibitions around substances be amended to exclude legal substances, thereby permitting individuals to use cannabis when they are prohibited from using other recreational substances.

This presentation will report three separate cases in Ontario, Canada, in which physicians sought amendments to substance use prohibitions set out by the Review Board to allow individuals to use cannabis legally (while still being prohibited from using other illicit substances). This presentation will explore the various arguments used to request such an amendment to an individual's disposition as well as the varying decisions made by Review Boards in response to these new requests.

This presentation will also discuss the "harm reduction" approach that is used as a basis in many of these recommendations to the Review Board. Specifically, this presentation will discuss the harm reduction literature around cannabis in the forensic psychiatry system.

This presentation seeks to review a shift in the Canadian forensic Review Board system following the country's legalization of cannabis in 2018, specifically that drug prohibition clauses are being amended to permit the use of cannabis for some individuals, where other substances remain prohibited. This presentation will aim to provide context for forensic professionals practicing in other jurisdictions that may have recently legalized cannabis or may do so in the future.

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### Cannabis, Legalization, Not Criminally Responsible



## I11 An Increasing Demand for the Psychological Evaluation of Asylum Seekers

Collin Lueck\*, Los Angeles, CA 90232

**Learning Overview:** After attending this presentation, attendees will learn: (1) the definition of “asylum” and the routes to obtaining asylum; (2) the details of the psychological evaluation of asylum seekers, including screening for symptoms, writing affidavits, and collaborating with legal organizations; and (3) the role that forensic psychiatrists can play in this process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by teaching attendees about the psychological assessment of trauma and mood symptoms in asylum seekers and about the current laws related to asylum.

There were more than 70 million forcibly displaced people (those who leave their home countries fleeing war or other conflict) worldwide at the end of 2018.<sup>1</sup> This number has risen steadily over the past ten years, driven upward by conflicts in Syria, other parts of the Middle East, and Sub-Saharan Africa. Some displaced people seek asylum in the country to which they immigrate, often when faced with deportation. Asylum refers to protection given to people who fear persecution in their home country due to their race, religion, nationality, political opinions, or membership in a particular social group.<sup>2</sup>

Most asylum applicants in the United States emigrate from China and Central and South America. The United States granted just over 26,000 asylum applications in 2017, according to the most recent data available. Those who are denied may face deportation.<sup>3</sup> As immigration has become a central factor in the polarized political climate of the United States, asylum regulations are rapidly shifting and are now an area of intense media scrutiny.<sup>4</sup>

In order to obtain asylum, applicants must demonstrate that they fear death or persecution and that this fear is “credible.” To demonstrate that their fears are well-founded, many asylum seekers will undergo psychological evaluations by psychiatrists, physicians of other specialties, or other mental health clinicians.<sup>2</sup> These professionals may write affidavits concerning the facts and psychological evidence related to persecution suffered by the asylum seeker. These affidavits can substantially affect the likelihood that an asylum application is granted.

Given the rising number of displaced people worldwide, as well as recent media attention given to asylum-seekers and other immigrants to the United States, professionals of multiple medical and mental health specialties may take interest in performing psychological evaluations and writing affidavits. It is important that this work be performed in a thorough, sensitive, and culturally informed manner. Although many asylum-seekers have experienced deeply traumatic events, their psychological responses to these experiences may be different than that traditionally seen in the United States.<sup>5-7</sup> Forensically trained psychiatrists can play a special role in performing evaluations, training others to do so, and raising awareness of the role of mental health clinicians in the asylum process.

This presentation will provide an introduction to conducting an asylum interview and will outline the key components that asylum evaluators should elicit during their examination. Those who attend will gain information about developing an asylum affidavit, and about the role that forensically trained psychiatrists can play in the asylum process during this global refugee crisis. In addition, attendees will gain a broader understanding of assessing trauma and mood symptoms in other patient populations.

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### Asylum, Trauma, Immigration



## I12 Recent/Proposed Legal Changes in Asylum Law and the Implications for Mental Healthcare

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**Learning Overview:** After attending this presentation, attendees will appreciate recent changes/attempts to change the political asylum process by the current administration. Attendees will learn about the potential impact of these changes/proposed changes on mental health providers and forensic practitioners who work with refugees and others seeking asylum.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by updating forensic practitioners on recent political events impacting those seeking political asylum in the United States. This presentation will also promote awareness of different levels of racism that may affect the treatment of minorities and disadvantaged people.

Mental health professionals play a key role in assisting applicants for political asylum to the United States.<sup>1</sup> Political winds during the current Trump administration coupled with unprecedented numbers of asylum seekers at the southern border have spurred recent attempts to fundamentally alter the asylum process. Specifically, this presentation will discuss: (1) Attorney General William Barr's July 29, 2019, order in "Matter of L-E-A," prohibiting asylum seekers from using the status of "immediate family member" as constituting a "particular social group" eligible for asylum in the United States; (2) the "Secure and Protect Act of 2019 (S.1494)," a Senate Bill sponsored by Senator Lindsey Graham introduced on May 15, 2019, which proposes changes that would, among other things, increase the amount of time children could be detained by their families and allow Border Patrol to quickly send unaccompanied minors back to Central America under certain circumstances.<sup>2,3</sup> This presentation will discuss the implications of these changes/proposed changes to asylum seekers and psychiatrists working on political asylum cases as well as the potential ill effects on mental health that these changes may have on asylum seekers, many of whom have already led lives affected by trauma.

A second goal of this presentation is to explore concepts of racism, including overt, covert (i.e., implicit bias), and institutionalized racism and debate whether the abovementioned changes represent a shift toward a more racist United States. It will be argued that policy changes like those presented above, while perhaps not inherently racist in themselves, potentially represent the institutional legitimization/codification of racist ideas. Increased awareness of the forms of racism that pervade our society will help ensure the fair treatment of individuals we treat and assess.

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### Asylum, Racism, Forensic Psychiatry



## I13 A Neurological Condition and Forensic Psychiatry: A Case Report

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**Learning Overview:** After attending this presentation, attendees will understand the concept of risk management with unconventional conditions. Attendees will also understand why clinical details are important, notably to make the proper diagnosis and offer solid evidence to the legal professionals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting a rare case report. As this clinical situation is not widely described, it will allow forensic professionals to be more familiar with the diagnosis-making process and how this can be defended in a court setting.

**Introduction:** Typically, individuals found to be not criminally responsible on the account of a mental disorder integrate the Canadian forensic system, due to suffering from a schizophrenia or bipolar disorder. Mitigating the patients' risk and promoting their rehabilitation are the focus of the forensic psychiatry team. The difficulties in achieving significant positive results and allowing patients to be released and later discharged into the community result from treatment-resistant psychiatric symptoms. Although this is an unconventional situation, this may occur when someone presents with a psychotic disorder due to a medical condition. Indeed, this medical condition may not have a good response profile to the medication usually used in psychiatry that deals with delusions and behaviors. Moreover, the evolution of this condition, or its day-to-day management, may be quite different from what is usually expected from a common psychiatric condition.

**Case Report:** Based on a case report, this presentation highlights the difficulty in providing a diagnosis of psychotic disorder due to a neurological condition in the forensic context. For instance, this relates to a case of multiple sclerosis with psychotic features. In this case, ruling out differential diagnoses, primarily psychiatric, was not an easy task due to the presence of a comorbid personality disorder. In addition, as multiple sclerosis extremely rarely presents with psychosis as its main symptom, the diagnosis was not easy to make. Moreover, due to the presence of delusional thinking in relation to the cause for the brain lesions, adherence to treatment and follow-up was poor.

**Discussion:** When someone is deemed not criminally responsible on account of a mental disorder in the court, it states that the individual's mental disorder is associated with deviant behaviors. The role of the Review Board in Canada (forensic system) is to make dispositions that will mitigate the risk and enhance rehabilitation. Making the appropriate diagnosis is essential to offer the optimal treatment response and assess its efficacy, notably on the risk profile. It is notably relevant in forensic psychiatry because evidence gets scrutinized, not only at the initial hearing, but also during upcoming hearings through the Review Board system. Moreover, inappropriate medical management will lead to risk for the patient (due to the progression of the disease inadequately treated) and risk for the community (due to the ongoing risk of safety). This case also shows how difficult risk mitigation is achieved and how risk formulation is performed because of an unstable neurological disease. Moreover, in this particular situation, the impact of propaganda that in this particular occurrence was detrimental to the patient's rehabilitation by increasing the patient's paranoid symptoms will be presented.

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### Neurological Condition, Propaganda, Risk Management



## I14 Differences Between Readmitted and Non-Readmitted Patients Discharged From Italian Psychiatric Security Facilities

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**Learning Overview:** After attending this presentation, attendees will know the characteristics of a group of psychiatric patients discharged between 2008 and 2018 from a psychiatric security facility in northern Italy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of important clinical aspects, especially the differences between patients readmitted and not readmitted after discharge.

There is substantial international literature on the clinical and criminological characteristics of patients discharged from forensic hospitals.<sup>1-3</sup> In Italy, unfortunately, there are few studies on this topic.<sup>4,5</sup> In order to fill this gap, this study gathered the clinical and criminal data of 543 male psychiatric patients discharged from the only secure psychiatric facility in the Lombardy Region of Italy between 2008 and 2018. Among this group of patients, the prevalence of readmission into the forensic facility is 11.6% with an average of 22.7 months after discharge. The prevalence of post-discharge violent offending in this population was 3.5% within an average of 28 months after discharge. This study focused on the differences between the patients discharged and not readmitted (first group) and the patients readmitted at least once (second group). This study found that the median duration of inpatient treatment was longer in the first group than in the second (28.2 vs. 19.6 months). It was then noticed that the average age at the time of discharge was higher in the first group (43.2 vs. 38 years old). Moreover, the prevalence of psychotic spectrum disorders as the primary diagnosis was higher in the not readmitted group (53% vs. 44%) and the prevalence of personality disorders as the primary diagnosis was higher in the readmitted group (30% vs. 44%). In regard to substance abuse, a known major risk factor for violent behavior, having substance abuse as a secondary diagnosis was more frequent in the second group (26% vs. 42%). As far as the type of index crime was concerned, the patients who were not readmitted were more likely to have committed violent crimes (homicide and attempted homicide). In summary, this study found retrospectively that patients discharged and readmitted into an Italian forensic psychiatric facility were younger at the time of the first discharge, had a shorter length of the stay, most likely had a personality disorder as a first diagnosis, and had substance abuse as a secondary diagnosis, compared to patients who were not readmitted during the study period.

**Conclusions:** Some clinical implications arise from these findings. First, they suggest that we should make a more accurate risk assessment and risk management in young patients with personality disorders and substance abuse, because these are the patients who will most likely be readmitted. Furthermore, in these types of patients, the length of stay should be considered as an important prognostic factor when evaluating the possibility of discharge.

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### Discharge, Readmission, Recidivism

## I15 Identification of Risk and Protective Factors for Violent Behavior in a Population of Forensic Psychiatric Patient Offenders in the Apulian and Lucan Areas of Italy

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**Learning Overview:** After attending this presentation, attendees will better understand risk and protective factors of violent behavior inside the forensic psychiatric facilities in Puglia and Basilicata, Italy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating, once identified, the risk and protective factors of violent behavior in socially dangerous subjects with specific treatment proposed.

**Introduction:** Over the past few years in Italy, the treatment of psychiatric offenders has undergone profound changes, following a series of court and legislative interventions that have radically changed the forensic psychiatric treatment panorama. The process of overcoming and closing judicial psychiatric hospitals (Ospedali Psichiatrici Giudiziari [OPGJ]), presented to the Decree of the President of the Council (D.P.C.M.) on April 1, 2008, with the transfer of health competencies related to detainees to the Ministry of Health over a period of less than ten years, led to an approach strongly based on the regionalization of the treatment of forensic psychiatric patients. This change involved, among other things, the establishment of the Residences for Execution of Security Measure (REMS) and the transfer to regional psychiatric facilities all patients with a profile of being socially dangerous in a psychiatric sense so as not to require a correctional-type security measure.

Law 81 of May 30, 2014, sanctioned the final transition to different treatment models for the socially dangerous offender. All Italian regions have therefore involved themselves in identifying and creating structures to welcome people deemed socially dangerous from the psychiatric point of view. At the same time, it has become a regional task to plan the social reintegration of acquittees with infirm minds coming from OPGs that are closing, whose social and health assistance have been entrusted to mental health departments.

This was also the case for the Puglia Region, where a special commission planned the discharge of the internees in the OPGs originating in the region, providing, in addition to the REMS already activated, ten dedicated communities (communities for patients with safety measures) for offenders with mitigated socially dangerous profiles.

In this regard, the welfare reorganization of the mentally ill offender presents a series of critical issues related to the estimation of the levels of social danger and the adequacy of the different structures for the treatment and rehabilitation of patients with specific profiles, as well as to the safety and protective needs of the community.

Considering the lack of empirical data in this regard, the acquisition of greater knowledge on risk and protective factors within the specific and particular Italian psychiatric-forensic treatment reality is essential.<sup>1-5</sup>

This study aims to evaluate the recurrence rate of new violent behaviors and/or crimes in forensic psychiatric patients subjected to a security measure and entrusted for treatment to mental health departments (DSMs) in any form of treatment (REMS, dedicated community, therapeutic-rehabilitative community, outpatient treatment, etc.) and to evaluate the effectiveness of treatments and the relative differences in reducing violent behavior.

**Conclusions:** The main expected result is the identification of risk and protective factors for aggressive and/or violent behavior within the specific forensic psychiatric population under study after the closure of high-security Italian hospitals. The second expected result is the estimation of criminal recidivism rates, as well as aggressive and violent behavior in the study population and the relative differences in comparison with the control group. The initial data obtained one and one-half year after the beginning of the present research show how aggressive and/or violent behaviors are present in the experimental group in only a negligible percentage. The rehabilitative approach with these types of patients seems to be effective.

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### Risk Factors, Protective Factors, Violent Behavior



## I16 Women and Men Who Committed Murders: Male/Female Psychopathic Homicides

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**Learning Overview:** The goal of this presentation is to examine gender differences in psychopathic homicides.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by redefining the concept of female psychopathy and, consequently, of the available diagnostic tools.

**Introduction:** The relationship between the psychopathic dimension and homicide is controversial. The origins of psychopathy and its psychopathological position are still controversial. This disorder is characterized by manifestations that involve the subject’s emotional drive and interpersonal sphere, as well as the lack of respect for other people’s rights and for social rules. This is an important risk factor for antisocial and violent acts. Moreover, this increases criminal recidivism and hinders therapeutic change.

Psychopathic homicide, according to the psychopath’s personality traits, are either committed to obtain a personal benefit (e.g., money, sexual opportunities, status enhancement) or they are a result of a sadistic and predatory behavior, which is frequently associated with substance abuse. Moreover, some gender differences can also be observed in the homicide phenomenon regarding victims, weapons, and psychopathic dimension.

**Methodology:** This study involved male and female murderers suffering from a mental pathology with excluded and partial criminal responsibility who had been interned in the Residence for the Execution of Security Measures (REMS) of Castiglione delle Stiviere, Puglia, and Basilicata as a security measure to prevent the risk of criminal recidivism, together with male and female prisoners of Calabria, Basilicata, and Puglia convicted of murder with full criminal responsibility from March 2015 through the end of 2017. The sample consisted of 39 female and 48 male perpetrators of homicide and attempted homicide.

The entire sample of male and female murderers was assessed by the Structured Clinical Interview for *DSM-5* (SCID-5) and the Personality Inventory for *DSM-5*—Informant Form (PID-5 IRF) (per the American Psychological Association [APA], 2015), which were recently validated for the Italian population to determine major psychiatric disorders and personality traits, respectively, and can also be used by an examiner. In addition, the validated Italian version of the Psychopathy Checklist-Revised (PCL-R) was used.

**Results:** This study analyzed the total scores of the PCL-R and scores for Factor 1 and Factor 2. In the overall analyzed sample, this study found an average score of 13.18 (standard deviation (sd): 7.19); in the male group, 12.29 (sd 6.36); and in the female group, 14.28 (sd 8.05). Therefore, in all cases, an average value was found that was below the threshold that defines the condition of psychopathy. Statistically significant was the difference between the two groups of homicide/attempted homicide committed by men or women in regard to the distribution of Factor 1 scores, which evaluated the interpersonal/affective aspects. Here, the total average score obtained from the entire sample was 6.65 (sd: 4.22). However, this study obtained an average of 5.54 (sd: 3.49) for men and a higher value for women, with an average of 8.02 (sd: 4.65).

The differences observed for Factor 2, which concerns the aspects of social deviance, were less significant. Therefore, the average in the total sample was 6.19 (sd: 3.77); in the group of men, 6.48 (sd: 3.77); and in the group of women, 5.85 (sd: 3.79).

**Conclusion:** This study allowed reflection on how psychopathic traits are expressed when they are associated with the maximum expression of social deviance: homicide and attempted homicide. Therefore, based on the results obtained, it is possible to state that: (1) contrary to expectations, from an epidemiological point of view, the psychopathic dimension in this sample was not pronounced in crimes such as homicide and attempted homicide; and (2) there are differences in the phenotypic manifestations of the psychopathic dimension in this sample between the male and female genders. In women, expressions of psychopathy are more closely related to interpersonal/affective disorders, rather than to antisocial behavior, which is more frequent in men.

Finally, it is important to point out that, although the PCL-R represents an accurate assessment tool for women as well as men, its theoretical construct would require a “feminine” review, especially for Factor F1, which concerns the affective-interpersonal manifestations of women. Indeed, in line with other international surveys, this research indicates the existence of gender differences. Therefore, some work is required to redefine the concept of female psychopathy and, consequently, of the available diagnostic tools.

### Murders, Gender, Psychopathic



## I17 Sex Offenders and Psychopathy: A Study of an Italian Sample

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**Learning Overview:** After attending this presentation, attendees will better understand the presence of psychopathy in subjects who have committed sexual offenses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying the presence of psychopathy in subjects who commit crimes of a sexual nature and will assess the possible influence on criminal recidivism.

**Introduction:** Sexual offenses are among the crimes that cause great social alarm. Thus, attention to intra- and extra-prison treatment perspectives for perpetrators of deviant sexual behaviors has increased in recent years.

In the face of legislation in force in Italy, attentive to respect and safeguarding the personal rights of the victims and severe with regard to those responsible for the abuses, the institutional initiatives aimed at the prevention of this type of crime seem to be few, other than that they can be characterized by very high, specific relapse rates, as is the case with programs and initiatives with treatment and re-socialization purposes.<sup>1,2</sup>

Violent antisocial behavior is characterized by typicality of thought, denial, and minimization of damage to the victim and suspension of empathic abilities. One of the persistent characteristics in sexual offenders is denial. Denial is a personality trait or a conscious or non-conscious strategy that the subject adopts to protect himself and is supported by different cognitive dimensions. When complete negation is not present, minimization is often present, (i.e., the subject can admit the crimes committed and his own responsibility, but minimizes other aspects, such as the seriousness of the consequences on the victim).<sup>3</sup>

This study has identified two types of negation: total negation and partial negation.<sup>4</sup> Minimization is subdivided into minimization of the aggression in terms of frequency and gravity, of responsibility, of damage, of premeditation, and of fantasies. The primary function of these mechanisms is self-protection, that is, to stop denying means to suffer social and legal sanctions, while denial allows the aggressor to safeguard an acceptable image before others, the family, and himself. Denial protects him from unpleasant feelings of shame, guilt, and self-denigration and can help save himself and his family from following the admission of guilt.<sup>4</sup>

Some authors have conducted research focusing on the cognitive distortions of sex offenders.<sup>5</sup> These authors define cognitive distortions as a system of beliefs that in sexual offenders would involve opinions about children, women, the world, and themselves. The justifications, perceptions, and judgments would be used by the offender to rationalize his behavior as a molester. These beliefs, moreover, would allow the maintenance of the sense of self-esteem and the avoidance of negative consequences, thanks to a shift of responsibility from internal causes to one's own deviant behavior, but also to reduce the discomfort felt (for having carried out an unacceptable behavior), the guilt, and the shame, with the outcome that this behavior can be repeated.

The evaluation, in particular, focused on the coping resources and strategies of offenders and on personality characteristics, particularly psychopathic. Scientific literature reports that psychopathic traits characterize criminal behaviors, especially the violent ones. Psychopathy is also considered a risk factor with respect to the recurrence of sexual crimes, even if the relationship between psychopathy and sexual violence is rather complex.<sup>6</sup>

In light of the data reported, a research project was started in collaboration with the Bari Prison and the Altamura branch aimed at identifying the possible presence of psychopathy in this sample. These are 16 definitive prisoners who committed sexual crimes that gave their consent to their involvement in the present.

The first data obtained show that the sample had a rather low average total Psychopathy Checklist-Revised (PCL-R) score ( $M=14$ ;  $SD=8.3$ ) with higher mean values for Factor 1 (Interpersonal/Affective) ( $M=7.68$ ;  $SD=4.78$ ) greater than the Factor 2 (Social Deviance) ( $M=5.5$ ;  $SD=3.89$ ). Among the most significant data obtained on the different items of the PCL-R was that it is possible to detect higher scores with regard to talkativeness and superficial charm ( $M=1.5$ ;  $SD=0.79$ ), the sense of grandiosity ( $M=2.62$ ;  $SD=5$ ), the need for stimuli and the propensity to boredom ( $M=1.3$ ;  $SD=0.84$ ), and the manipulative style ( $M=1$ ;  $SD=0.86$ ). Also relevant are the scores of items regarding emotional and sexual relationships: promiscuous sexual behavior ( $M=1.56$ ;  $SD=2.91$ ) and numerous short-term relationships ( $M=2.3$ ;  $SD=3.6$ ).

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### Sex Offender, Psychopathy, Risk Factors





## I18 Sexual Addiction and Deviant Sexual Behavior: Are They Comorbid Disorders?

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**Learning Overview:** After attending this presentation, attendees will be aware of the co-occurrence of sexual addiction and sexual deviant behavior. Attendees will know about the therapeutic strategies and how to address these conditions in clinical practice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by looking into a topic that has been understudied but has the potential to help better rehabilitate sexual offenders.

Sexual behavior is part of human behavior and naturally exists among each human being. Societies have drawn a lot of concerns in relation to sexuality, even when it relates to non-deviant sexuality. Mental health clinicians are confronted with addressing this topic as sexuality has the potential to cause a lot of distress. The main sexual disorders that have been studied for decades are hypersexual behavior and deviant sexual behavior.

Although these two sexual behaviors appear to be of different types, some studies highlighted that they may be comorbid. Indeed, it is not uncommon to identify individuals with a paraphilic disorder who also present with hypersexual behaviors.<sup>1</sup> In addition, some individuals with addictive sexual behavior tend to develop non-conventional, deviant, and/or illegal sexual behavior as a means to increase their sexual excitement (voyeurism, exhibitionism, and rape, for example). Also, epidemiological studies have shown that two-thirds of patients suffering from sexual addiction also suffer from a paraphilic disorder.<sup>2</sup>

For some sexual offenders, focusing on sexual behavior is interpreted as a way for them to cope with their psychological difficulties, both cognitive and emotional, which may also be explained by the fact that they present with a sexual addiction. North American studies showed that 35% to 55% of individuals who have sexually offended suffered from a sexual addiction.<sup>3,4</sup> In addition to finding that these two conditions are present among the same population, studies have shown other psychopathological similarities, such as personality disorder, relationship difficulties, cognitive distortion, and other types of addictions.<sup>2</sup> Therefore, if these two conditions may be comorbid, it becomes relevant to look into common therapeutic interventions (assessment tools, types of therapeutic programs, attitude of the clinicians, and expected efficacy).

The principle aim of this presentation is to offer a screen shot of the current scientific knowledge related to sexual addiction and sexual violent behavior as comorbid conditions, in terms of their common clinical signs and therapeutic interventions. Clinical vignettes will be used to highlight the data of the literature review and the possible therapeutic options.

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### Addiction, Violence, Sexuality



### I19 The Personality Assessment Inventory (PAI): Treatment Scales and Interpersonal Characteristics in a Sample of Men Charged With or Convicted of a Sexual Offense

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**Learning Overview:** The goal of this presentation is to inform those who are involved in the assessment and treatment of persons who have sexually offended of considerations related to treatment prognosis and personality characteristics.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the ability of evaluators of sexual offending behavior and treatment providers to make treatment recommendations based on information obtained from psychological testing.

This presentation will focus on the relationship between the Personality Assessment Inventory (PAI) interpersonal personality traits of dominance and warmth and PAI scales associated with treatment motivation and process.<sup>1</sup> The PAI Treatment Rejection (RXR) scale assesses treatment motivation levels with low scores indicating high motivations for treatment and high scores indicating low motivation for treatment. However, since motivation for treatment does not always equal a positive outcome, the PAI Treatment Process Index (TPI) is composed of 12 features that indicate levels of obstacles that may be present within treatment, such as hostility, low motivation, defensiveness, and low social support, as well as correlate highly with withdrawal, hostility, and alienation. It was hypothesized that RXR and TPI would be negatively associated with the PAI scale assessing Warmth (WRM) and positively associated with the PAI scale assessing Dominance (DOM). In other words, it was expected that individuals with lower levels of WRM and higher levels of DOM would be less motivated for the treatment process and were predicted to experience a greater amount of challenges within treatment.

Data were collected from 101 men who underwent comprehensive evaluations of sexual behavior after being charged with or convicted of a sexual offense or sexual boundary violation. After invalid PAI data were removed, the final sample was 92 men who ranged in age from 19 to 80 years old ( $M=44.84$ ,  $SD=14.79$ ). The current presentation will discuss the relationship between several PAI scales and the relationship between the PAI scales and number of prison disciplinary infractions, age of examinee, self-esteem rating, victim(s)' age and sex, history of parental mental illness, substance abuse, and criminal offending; prior mental health or substance use treatment; and prior sexual behavior treatment. This presentation will assist clinicians who perform assessment and provide treatment to individuals with problematic sexual behavior in using the PAI and other psychological testing in making treatment recommendations.

**Reference(s):**

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**Sexual Offending, Personality Assessment, Treatment**



## I20 Frontotemporal Dementia and Sex Offending: Neurological Neuropsychiatric and Legal Issues

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**Learning Overview:** The goal of this presentation is for mental health professionals and those involved in the criminal justice system to recognize how the neurological disorder Frontotemporal Dementia (FTD) can be a factor in new onset sexual offending in middle-aged and older subjects

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a basic understanding of FTD. Attendees will have greater sensitivity to the presence of this condition in first-time middle-aged, and older offenders, will learn how to use expert witnesses more effectively in such cases, and will have a basic understanding of diagnostic methods.

FTD is an increasingly recognized brain disorder that begins to manifest in the mid-40s. Second only to Alzheimer's disease in prevalence, FTD accounts for 20% of early onset dementia cases. The signs and symptoms of FTD often go unrecognized as a brain disorder and may be mistaken for psychiatric symptoms.

Neurodegenerative diseases can affect executive function (i.e., higher-level decision making, social emotional processing, and self-awareness resulting at times in inappropriate sexual and violent behaviors). This can lead to antisocial and criminal behaviors that first appear in middle-aged individuals or in later life.

This presentation aims to educate attendees how the behavioral variant of FTD (bvFTD) is of compelling medical/public health and jurisprudence relevance. FTD is often missed by psychiatrists and neurologists. The predominant symptoms being social, emotional, and behavioral makes it less likely to be recognized as a neurological disorder; given the lack of overt cognitive problems, unlike other forms of dementia such as Alzheimer's, psychiatrists may often misdiagnose it for bipolar disorder or some form of impulsive control disorder.

Six clinical features identify bvFTD: (1) disinhibition; (2) apathy/inertia; (3) loss of empathy; (4) perseverative and compulsive behaviors; (5) hyperorality, especially of sweets; and (6) dysexecutive neuropsychological profile. At least three features must be present to diagnose possible FTD.

BvFTD does not have to show overt cognitive impairment (i.e., intellect, reasoning, memory). What that means is that individuals with early bvFTD may continue to work, but only become behaviorally and emotionally unstable.

A 2015 University of California San Francisco retrospective medical record review study found that within the subgroup of FTD, more than a third of people had acted out with criminal behaviors.<sup>1</sup> A 2002 study of Swedish court-ordered forensic psychiatric evaluations noted that sexual offenses were most typical of older offenders with dementia. Hypersexual behavior may be present in as many as 20% of those with bvFTD.

Hypersexuality and inappropriate sexual behavior may be the first symptom of the disorder within a subgroup bvFTD. This hypersexuality is not believed to occur purely from poor impulse control or frontal disinhibition but from alterations in the sexual drive from lesions in the right temporal and limbic areas. These sexual behaviors can include new onset involvement in pornography, including child pornography, voyeurism, chatlines, prostitutes, and hands-on sexual behavior and public masturbation. Temper tantrums, binge eating, and lack of empathy to people and animals may be concurrently present.

Given the age of onset (i.e., in mid-40s), FTD may manifest in the prime of professional and family life. Sexual misbehaviors that emanate from FTD can have devastating consequences on individuals and families. This is complicated by the fact that individuals with FTD may appear cognitively intact *and even acknowledge* that their behavior is inappropriate but appear to lack remorse and control over their actions.

Establishing bvFTD as a cause or a factor in criminal actions may be exculpatory or mitigating. Instead of being identified and punished as sex offenders, subjects with neurodegenerative diseases, such as bvFTD, could be treated differently by the legal system. For example, providing appropriate neurologic evaluation and channeling subjects to palliative and medical institutions.

Dysfunction of the orbitofrontal and ventromedial cortex may be identified by neuropsychological testing. Routine neuropsychological batteries are not adequate. Tests such as the Iowa Gambling Task and tests used from autism/moral reasoning/theory of mind tests such as Faux Pas Recognition Test may be more helpful.

Neuroimaging can be helpful in evaluating new onset criminal behavior in adults and should always be considered. Positron Emission Tomography (PET) scans in the early stages and by Magnetic Resonance Imaging (MRI) scans in later stages can help identify FTD and separate it from psychiatric disorders such as bipolar disorder. PET can help with the diagnoses of bvFTD and has recently been authorized for use by Medicare.

Among the subjects covered in this presentation are: educating attendees on the neurological and neuropsychiatric aspects of bvFTD; the evaluative process and management of such cases; the relevance of specialized psychological and physiological testing (penile plethysmography and polygraph) in such cases; discussion of the role of expert witness use and testimony in such cases; and a prosecutor's perspective on dealing with sexual criminal defendants with brain disorders.

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### Frontotemporal Dementia, Behavioral Variant, Sexual Offending



### I21 When Crime and Illness Overlap: California's New Mental Health Diversion Plan, Assembly Bill 1810

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**Learning Overview:** After attending this presentation, attendees will be familiar with: (1) recently enacted California Assembly Bill (AB) 1810 that implements Mental Health Diversion; (2) the primary purpose of this legislation concerning the disproportionate representation of persons with mental illness in jail; (3) evidence and opinions for and against AB 1810; and (4) suggested recommendations for community mental health professionals working with this specific population.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community in several ways. It will expand clinicians' knowledge and interpretation about a relatively new California mental health diversion law. In addition, this presentation will discuss existing programs designed to treat those individuals who qualify under this new law and identify the need that will arise for the development of treatment programs across a continuum of community care.

Assembly Bill 1810, also known as "Mental Health Diversion," was implemented in June 2018. This bill was in response to a bed space shortage at the California Department of State Hospitals (DSH) and jail overcrowding due to the growing population of persons with mental illness. The DSH shortage was attributed to an increasing population of those found "Incompetent to Stand Trial (IST);" namely, there was a 60% increase from 2013 to 2018 of individuals deemed IST and sent to state hospitals. Moreover, it was estimated that from 2010 to 2017, there was an 86% increase in the number of inmates in the Los Angeles County jail system with mental health problems.

Although AB 1810 promotes three major changes, it also incorporates a broader perspective: (1) increased diversion of individuals with mental disorders to mitigate their entry and reentry into the criminal justice system while protecting public safety; (2) allows counties discretion and flexibility in the development and implementation of diversion for individuals with mental disorders; and (3) provides diversion for this population across a continuum of care settings that addresses their unique mental health treatment and support needs.<sup>1</sup>

Proponents of the bill cite that about one-third of inmates in California's jails suffer from serious mental illness.<sup>2</sup> They highlight that prior to AB 1810, California criminal courts had limited options to rehabilitate persons with mental illness charged with felonies.<sup>2</sup> This bill allows the court to order mental health treatment, counseling, and medication adherence for those whose mental illness played a role in the alleged misdemeanor or felony offense. However, certain criminal charges preclude an individual from consideration under this bill; that is, murder, manslaughter, rape, and other sex offenses.

Opponents of the bill express a number of concerns focused primarily on unclear and vague operational details. The roles and collaboration between courts, law enforcement, and community mental health treatment providers are not well defined. Further, counties may not be required to create or pay for new treatment facilities or programs, thus relying on pre-existing resources in community-based mental health treatment.<sup>2</sup> Care will potentially default to community psychiatric facilities and providers who may not be trained or willing to work with this unique forensic population.

This presentation will not only discuss AB 1810 but will also highlight its impact on those with mental illness who are charged with crimes, community mental health systems, the criminal justice system, and the public. Recommendations will be discussed for ways to deliver the necessary mental health services and structure for this unique population.

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#### AB 1810, Diversion, Felony



### I22 False Allegations of Sexual Abuse and Malpractice in Child Custody: A Big Court Case in Italy

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**Learning Overview:** After attending this presentation, attendees will be more skilled in how to assess a malpractice scenario regarding child custody and allegations of sexual abuse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting solutions that could prevent malpractices scenarios in child custody settings and allegations of criminal child abuse.

This presentation will take the Case of Bibbiano, a small city in the center of Italy into the Emilia Romagna region, as an icon of malpractice. Code named “Angels and Demons,” this case presented a disturbing picture of psychological violence toward children and their parents. Seventeen people, including medical doctors, social workers, and psychologists, were arrested by the Italian police. They are accused of submitting false reports regarding the family situation of children and also of having modified their drawings by adding genital attributes. Finally, they are accused of having brainwashed children with false memories of abuse.

Despite not yet being confirmed, these facts had a huge impact on the international press, causing questions about the entire Italian child custody system, allegations of sexual abuse system, and the competence of Italian psychologists, social workers, and medical doctors.

The crucial point of this presentation will be to clarify the Italian guidelines about child custody practices, about cases of allegations of sexual abuse, and the differences between them, in addition to clarifying what happened in the “Angels and Demons” case.

Italian legislation applies child custody to foster families or group homes only as a residual solution, and not as the final choice as happened in Bibbiano. Also, according to law number 172/2012 of the Italian penal code, in criminal offenses involving minors, the presence of an expert who acts as an assistant to the judge is required, but the assessment of sexual abuse always belongs to the judge him/herself. Despite what allegedly happened in Bibbiano, the expert has no legal status to decide whether a sexual abuse occurred or not.

Following what law n. 24/2017 of the Italian penal code states, the expert has to follow accredited guidelines, such as “La Carta di Noto,” the Italian national guidelines for child abuse allegations and can be neither suggestive nor manipulative during child interviews. These guidelines are totally antithetical to the presumed facts of the Bibbiano case where experts brainwashed children and used therapy to create fake abuse memories. Overall, it appears extremely important that the judge could ask the expert witness to use the standard methods for this type of case.

Next, the Juvenile Court and social services must build intervention systems, aimed at promoting residual family resources, to allow the child to live in a context that could prevent risks to his psychological development. Moreover, in cases of alleged abuse or maltreatment, law n.154/2001 has provided that the suspect should be removed from the family unit instead of removing the minor, as seems to have happened in the Bibbiano case.

It is hoped that in the future, the Juvenile Court, starting from these facts, rather than delegating to the social services will involve technical experts with specific training on the assessment of parenting skills. It is also hoped that, through this presentation, awareness of the need for specific training and specific technical skill may grow, beginning with social workers and psychological experts, up to judges themselves.

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#### **False Allegations of Sexual Abuse, Malpractice, Child Custody**



## I23 Brain Imaging in Death Penalty Mitigation

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**Learning Overview:** After attending this presentation, attendees will have learned about various types of brain imaging and the evolving application in criminal cases and will have a better understanding of how to be sensitive to the possibility of mitigation and the appropriate use of experts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing understanding of the possibilities, use, and misuse of brain imaging techniques as they apply to criminal defenses.

Brain imaging techniques, such as Magnetic Resonance Imaging (MRI), Computed Tomography (CT) scans, functional MR imaging, diffusion tensor imaging, perfusion imaging, Positron Emission Tomography (PET), and Single-Photon Emission Computed Tomography (SPECT), have increasing applications in clinical settings

Increased reporting in the peer-reviewed literature and clinical evidence has demonstrated sensitivity and specificity for even the more recent techniques, such as Diffusion Tensor Imaging (DTI). In regard to its use in evaluations of Traumatic Brain Injury (TBI), Hulkower noted, “Despite significant variability in sample characteristics, technical aspects of imaging, and analysis approaches, the consensus is that DTI effectively differentiates patients with TBI and controls, regardless of the severity and timeframe following injury. Furthermore, many have established a relationship between DTI measures and TBI outcomes.”<sup>1</sup>

Increasing advances in brain imaging will compel the legal system to determine which imaging techniques have probative value from prejudicial and misleading ones.

The track record for the success of functional neuroimaging in exculpating specific-intent crimes, such as murder, is not a good one. However, presentation of functional brain imaging has fared better in the mitigation of the death penalty in some capital offenses.

In November of 2002, just five months out of prison, 32-year-old Coy Evans was charged with first-degree murder of a police officer, two counts of armed robbery, armed burglary, kidnapping, and possession of a firearm by a convicted felon. Evans was later convicted of fatally shooting Sgt. Dale Green, a 13-year veteran of the Tallahassee police department, while the officer was investigating a home-invasion robbery. The jury spared Evans from the death penalty. A Single-Photon Emission Computerized Tomography (SPECT) brain scan may have been a factor in the jury’s decision

Approximately 44% of the neurobiological (genetic/neurological) claims raised were attempts to mitigate sentencing.<sup>2</sup> Failing to adequately investigate the probability of a brain abnormality may constitute ineffective assistance of counsel. Failure to obtain brain imaging studies in a first-time older offender may be an example, given increasing evidence that the behavioral variant of frontotemporal dementia may be a factor in their offending.

The defense (in plaintiff cases) and the prosecution (in criminal cases) have generally been successful at portraying much brain imaging evidence as “junk science.”<sup>3</sup> However, in 2018, the United States District Court for the Southern District of Florida ruled that DTI satisfied the *Daubert* standard for admissibility in *Marsh v. Celebrity Cruises, Inc.*, a personal injury claim for mild traumatic brain injury. The Court concluded that a lack of a standardized protocol for the acquisition and interpretation of DTI results did not make DTI technology “junk science” nor render the opinions of a board-certified neuroradiologist unreliable. In arriving at their opinions, the Court appears to overlook elements of *Daubert*, (i.e., known or potential error rate; the existence and maintenance of standards controlling its operation; and possibly whether it has attracted widespread acceptance within its relevant scientific community). This lack of adherence to established evidentiary standards has not stopped the increasing use of DTI in the courtroom.

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4. *Marsh v. Celebrity Cruises, Inc.*, Case No. 1(17-CV-21097-UU).

### Brain Imaging, Mitigation, *Daubert*



### I24 You Saved My Butt: A Change in Jurisdiction for a Threat Against a Judge, and Why Forensic Psychiatrists Exist

Vivian Chern Shnaidman, MD\*, Princeton, NJ 08540

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**Learning Overview:** After attending this presentation, attendees will understand the myriad of issues that occur when mentally ill plaintiffs and defendants are let loose in the civil arena as *pro se* litigants. Attendees will understand how the legal world, as well as the rest of the medical world, is sorely lacking in the understanding of the mental health field. Through the detailed analysis of one specific custody case, including the threats to one judge's life and the resulting change in jurisdiction of the trial that did not lead to new charges, attendees will begin to identify every step at which potentially dangerous individuals may be diverted toward treatment, and every juncture where a forensic psychiatrist might "first do no harm." The ethical, moral, and legal implications of our field will be discussed within the framework of one real case.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the many opportunities forensic psychiatrists and psychologists have to intervene in the trajectory toward dangerousness. This case illustrates the long-term decompensation of one individual, his multiple encounters with both the medical and the criminal justice systems, and every instance in which his mental illness was ignored. It culminated with a death threat against a judge that was handled via a change in venue, which could have resulted in the death of that judge. This case is a perfect teaching case to help the forensic mental health and legal communities understand our roles in preventing acts of violence and could theoretically result in new legislation.

The case of DS began as a routine family spat. DS claimed to be educated and appeared to be intelligent. When he could not hold a job and did not appear to behave like other young husbands, his wife asked him to please go for counseling. His refusal led to a series of unfortunate events, culminating in a divorce. As time went on, DS began to behave in more and more bizarre ways. Despite the numerous episodes of imminent danger to his children and the existence of one reliable parent, the laws surrounding the requirements of equitable distribution of parenting time led to the courts permitting DS to have ongoing access to his two very young children and to continually put them in harm's way.

This case presentation will examine the story of this case and the legal system that ignores mental illness at every turn. Attendees will also see how other medical specialties minimize and marginalize psychiatry, how psychiatric illness is misunderstood, and how, when this case culminated in a clear and convincing threat to a judge's life in open court, this judge's only recourse was to send the case to another jurisdiction. (What she said in the title was only the punch line, years later!)

Cases similar to this one enter the legal system every day and are disposed of in similar ways, often resulting in much more catastrophic outcomes. Some will be presented via their published decisions as a part of this presentation. This presentation will include discussion about how to inform both the legal community as well as the general population via the media about every opportunity to stop meaningless violence by addressing mental illness in the community. This case is a fantastic example; because of every dropped opportunity, it illustrates and offers the chance to brainstorm some ideas for the future, supporting the theme of this year's meeting, *Crossing Borders*, where we may finally cross the border between psychiatry and law and bring the two disciplines together in a way that would truly serve society as well as individuals.

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#### Death Threat by Mentally Ill, Change in Venue, Prediction of Dangerousness



## 125 The Influence of a Juror's Note-Taking on Other Jurors' Memories for Testimony

Caitlin A. Pratt\*, Edmond, OK 73034; Jaclyn K. Maass, PhD, University of Central Oklahoma, Edmond, OK 73034

**Learning Overview:** The goal of this presentation is to discuss how jurors who take notes during a trial may be able to heavily influence the opinions of jurors who did not take notes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding to the limited body of literature that exists on this topic and by bridging theories from the fields of forensic science, cognitive psychology, and social psychology.

This study seeks to investigate how a juror's memory for testimony may be influenced by a fellow juror who took notes during the trial. The non-note-taking juror may rely on the memory of the note-taking juror. If that is the case, could this juror's memories actually be altered? Research suggests that being exposed to misinformation after an event can alter a person's memory of that event, especially if it comes from a "reliable" source.<sup>1-3</sup> In addition to unconsciously altering memories, we sometimes choose to conform (i.e., change our opinions based on what others around us believe). When our decision is based on evidence provided by others, this is known as informational influence.<sup>4-5</sup> This influence may be of particular importance in jury deliberations.

Some research specific to jury settings does suggest that jurors may be influenced by opinions of other jurors who have taken notes.<sup>6-7</sup> However, these studies are relatively dated, and further research is needed to investigate whether a juror's actual memory of the testimony is influenced or just their judgement of the verdict.

This study will see how the opinions of a juror who takes notes during a trial influences the opinions of other jurors. Attendees will watch a video of a witness testifying at a mock trial. They will be sent to "deliberate." A confederate will have notes with inaccurate details about the testimony. Attendees will be tested again on their memories to see if the participant incorporates the other juror's inaccurate details. It is hypothesized that attendees' verdicts will be changed by the opinion expressed by the note-taking confederate, and that attendees' memories of the trial's details will be changed.

Jurors may believe that by choosing to trust the opinions of someone who has written details from the trial, they are more likely to have an unbiased, accurate memory of the trial. It is important that individuals know what outside sources may affect their memories and opinions, and this study will provide additional information in this field.

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1. Gabbert, Fiona, Amina Memon, Kevin Allan, and Daniel B. Wright. Say It to My Face: Examining the Effects of Socially Encountered Misinformation. *Legal and Criminological Psychology* 9, no. 2 (2004): 215-27.
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### Psychology, Note-Taking, Jurors





### I26 The Evaluation of Psychological Trauma of Individuals Who Escaped From Turkey Due to Human Rights Violations: Post-Traumatic Stress Disorder (PTSD)

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**Learning Overview:** After attending this presentation, attendees will be informed about the impact of psychological trauma on a well-educated group of people who migrated to Germany from Turkey.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the human rights violations and migration in Turkey over a well-educated group of people who migrated to Germany from Turkey.

**Introduction:** Post-Traumatic Stress Disorder (PTSD) shows specific symptoms and may arise after witnessing traumatic events, including death or death threats, serious injury, or encountering a situation that threatens the physical integrity of the individual. The latter is one of the most common causes of PTSD. One of the most common reasons for disrupting physical integrity is the pressure exerted on citizens by repressive states or regimes. After the 2016 coup attempt in Turkey, thousands of public officials (teachers, academics, doctors, engineers, etc.) were dismissed or imprisoned. A large number of well-educated citizens escaped from Turkey and sought asylum in other countries. Germany is a favored destination for Turkish asylum seekers. Since 2015, approximately 40,000 Turkish citizens have applied for asylum in Germany. The aim of this study was to analyze PTSD in Turkish asylum seekers in Germany and to discuss the obtained results.

**Materials and Methods:** Turkish citizens aged 18 years and over who sought asylum in Germany after the failed coup d'état in Turkey in 2016 were included in this study. Recruitment was performed via announcement in a mobile messaging group that functions as a support group for Turkish citizens in Germany. One hundred ninety-nine people responded to the announcement. The PTSD Checklist for *DSM-5* (PCL-5) scale, a questionnaire consisting of 25 questions (5 of them related to demographic information), was used to measure PTSD. Google® Forms was used to present the questionnaire in a survey application, which was sent to the study participants via mobile messaging applications. A descriptive analyses on the obtained data was conducted.

**Results:** One hundred ninety-nine people completed the questionnaire. 119 participants (59.8 %) were male, and 80 (40.2 %) were female; 118 participants (59.3 %) were in the age range of 26 to 35 years; 174 participants (87.4 %) were married, 25 (12.6 %) were divorced or single; 113 participants (56.8 %) have been in Germany for 7–19 months prior to participation in this study, while 15 (7.5 %) have been in Germany for 6 months and less; 177 participants (88.9 %) scored more than 33 points on the PCL-5 scale and were preliminary diagnosed with PTSD, while 12 participants (6.0 %) scored 22–33 points.

**Discussion:** PTSD is the most common type of psychiatric disorder after physical and mental trauma. In this study, the ratio of PTSD cases was higher than in similar literature. This may be a consequence of enduring repressive practices in Turkey or during migration. In order to minimize trauma and to provide rapid rehabilitation, it is important to develop new policies considering PTSD in rehabilitation practices for refugees. As the study only included a relatively small number of participants, additional studies are needed to draw definite conclusions and to avoid sampling bias.

#### PTSD, Detention, Refugees



## 127 The Forensic Analysis of the State of Health in Prisoners: Is It Possible to Prevent Psychiatric Diseases and Suicide Risks in Prison?

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**Learning Overview:** After attending this presentation, attendees will understand the importance of using a protocol that can prevent psychiatric diseases in prison and reduce the suicide risk in the prison population.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of surveillance systems in order to reduce the prevalence of psychiatric diseases and suicide in prisoners.

There are about 10 million prisoners in the world, of which 6 million are held in United States prisons.<sup>1</sup> From 2016 to 2018, there was a reduction in Europe in the detention rate estimated at 6.6%, while in Italy the prison population is growing: this phenomenon is linked to the increase in organized crime. As of June 2019, there are 23,442 prisoners in Italian prisons.<sup>2</sup> Due to the worldwide importance of the phenomenon in the public health, it is necessary to investigate the relationship between detention and prisoner health as well as the change in the psychophysical state of a subject after a period of incarceration. The scientific literature shows that in prisoners, there is a higher incidence of psychiatric diseases compared to the general population.<sup>3</sup> A systematic review of the literature shows that the prevalence of depressive disorders in prisoners is higher by 10%–12 % and almost half are affected by personality disorders. Suicide is the main cause of death among prisoners, and it causes over half of the deaths in prisons. The main risk factors are alcohol or drug abuse, family conflict relationships, and low socio-cultural status.

A review of the scientific literature was performed. The results were compared with the cases of Institute of Legal Medicine of Catanzaro, consisting of 30 forensic medical reports relating to the health of prisoners and their compatibility with detention. Prisoners were subjected to medical history collection and clinical examination. The clinical diary, laboratory, and/or instrumental investigations were analyzed. The most frequent pathologies were: anxious-depressive disorder (50%), gastrointestinal diseases (50%), osteoarticular diseases (40%), endocrine diseases (40%) and migraine (30%). The anxious-depressive disorder is reactive in all of cases and is a consequence of the detention and change of daily routine. Furthermore, gastrointestinal disorders and migraine may be expressions of an important anxiety somatization.<sup>4</sup>

In order to reduce psychiatric disorders and suicide risks, it is necessary to perform a medical and psychological evaluation for each new prisoner. In this way, it is possible to know the health status of each prisoner and to schedule a periodic monitoring of the diagnosed diseases. This evaluation requires specialized medical personnel able to set up an appropriate treatment. This study shows that prisoners suffering from psychiatric diseases are subjected to drug therapy without a complete diagnosis. It is important to adopt strategies in order to safeguard the mental health of prisoners. A mental health management protocol is not yet adopted in prisons worldwide. First, a risk stratification of the development of psychiatric diseases (for healthy subjects) or suicide risks (in subjects with known psychiatric pathology) must be conducted. A careful case history is needed, with investigations about the age of the subject, history of alcohol or drug abuse, familiarity with psychiatric pathologies, previous suicide attempts, type of relationship with family (a subject isolated from his/her own family has a higher risk of developing psychiatric pathologies). Once the medical chart of each prisoners is completed, it is necessary to schedule periodic updates to assess any worsening of health conditions. Furthermore, it is essential to train prison custodians in the recognition of signs of mental suffering (feelings of guilt, shame, despair, isolation). Periodic meetings must be scheduled between prison police and prison medical staff. During the meetings, updates concerning each prisoner are reported. The application of this protocol could reduce suicide episodes and allow for the early identification of risk signals.

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### Forensic Science, Prison, Mental Disorders



### I28 The Charleston Mother Emanuel African Methodist Episcopal (AME) Church Shooter: Debating the Psychiatric Experts' Competency to Stand Trial Reports

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**Learning Overview:** After attending this presentation, attendees will gain experience in approaching complex competency to stand trial cases by participating in a debate and discussion of the evaluation reports by psychiatric experts in a mass murder trial. Attendees will investigate a racially motivated perpetrator and gain an understanding of arguments for and against adjudicative competence. Additionally, relevant literature and case law involving competency to stand trial will also be incorporated into the discussion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by investigating a racially motivated perpetrator and will further understanding of arguments for and against adjudicative competence.

Psychiatric experts provide critical information to the trier of fact in assessing competence to stand trial.<sup>1</sup> How do opposing experts examine the same information and reach different conclusions regarding psychiatric diagnoses and opinions about competency? In this complex case, how an autism spectrum disorder may impact competency to stand trial is explored. There is developing case law and literature addressing individuals with autism spectrum disorders who perpetrate violence and engage in criminal behavior.<sup>2</sup> Diagnostic differences between severe personality disorders and primary psychotic disorders are explored in relation to their potential impact on competence. Young adults with social difficulties that could be suggestive of prodromal psychotic symptoms may be particularly difficult to evaluate with regard to the extent their symptoms interfere with the ability to work with an attorney on a defense strategy. Questions remain regarding how much ability constitutes sufficient present ability to consult with an attorney.

This presentation will describe the church shooting that occurred at the Mother Emanuel African Methodist Episcopal Church in Charleston, SC, and provide context for Mr. Roof's history, psychological functioning, and psychiatric symptoms. The three competency reports authored by two psychiatric experts and the psychological evaluation conducted on Mr. Roof will be reviewed and their findings will be debated. Additionally, Mr. Roof's desire to represent himself in trial and how this impacted the opinions regarding his competency to stand trial will be explored. Finally, relevant literature and case law involving competency to stand trial will also be incorporated into the discussion.

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#### Competency to Stand Trial, Racially Motivated Crime, Court Experts



## I29 Competence in Competence: Myths, Misconceptions, and Avoiding Pitfalls

George D. Annas, MD\*, State University of New York Upstate Medical University, Syracuse, NY 13202; Corina Freitas, MD\*, Syracuse, NY 13202

**Learning Overview:** The goals of this presentation are to improve knowledge and expertise in competence to stand evaluations and improve knowledge regarding common myths and misconceptions of Competence to Stand Trial (CST) evaluations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing those in the forensic field outside of mental health a better understanding of competence evaluations and improved skill in navigating interdisciplinary interactions in these areas.

Competency hearings in criminal proceedings are among the most common of the forensic mental health examinations, and the numbers are on the rise.<sup>1</sup> But what exactly occurs when a CST evaluation is ordered for a criminal defendant? Many in the field of forensic mental health regularly perform these types of evaluations for the courts, making them seem “simple” or “easy” for those who do this on a day-to-day basis. However, it can be easy to lose perspective and expect that all of the court officers are experts in this matter as well.

Even more frustrating can be interacting with an attorney who reaches a roadblock in dealing with a client and concludes the person must be “crazy.” In fact, experts in the mental health field find it surprising to learn that attorneys often do not understand the core concepts behind a defendant’s CST. This is often due to the fact that competency hearings are relatively rare occurrences and, therefore, few have regular experience with them. Since mental health experts are the ones doing these evaluations, perhaps this presentation can share some knowledge regarding how the process is approached and hopefully shed some light on the core concepts involved.

However, we should not forget that many of our colleagues have difficulty with these evaluations as well. Evaluating a criminal defendant’s CST can often seem so routine and mundane that some can forget the degree of skill it takes to perform these examinations adequately. It is not uncommon to come across CST reports that are inadequate in their conclusions, poorly worded, and poorly reasoned. However, even experienced evaluators are not always immune to making critical errors in this process, especially in the face of difficult interviews.<sup>2</sup>

Through case scenarios, sanitized from actual evaluations from firsthand experience, this presentation will address some common but challenging scenarios that can arise during these interactions with defendants. Examples will include, but are not limited to: how to deal with the defendant who says “I don’t know” to every question, how to differentiate delusions from incidental bizarre utterances, and some of the strategies one can use to determine when a defendant with a mental illness is “unwilling” vs. “unable” to participate in the evaluation. Also addressed will be a basic review and understanding of malingering and some of the ways in which this can be detected in this type of examination. Also addressed will be some proposals aimed at helping to prevent mistakes that are common among trainees and some that even experienced evaluators may fall prey to.

This presentation seeks to both provide a unique perspective in this “bread and butter” part of the world of forensic mental health and to hear the perspectives of those in the legal field.

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### Competence to Stand Trial, Criminal Competence, Forensic Psychiatry



### I30 Schema-Focused Therapy (SFT) in Forensic Patients With Personality Disorders: A Theoretical Model and Recommendations for Best Clinical and Preliminary Findings of a Multicenter Randomized Clinical Trial in the Netherlands

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**Learning Overview:** After attending this presentation, attendees will understand, from a psychotherapy model, a forensic modification of SFT and will be equipped to make recommendations for its implementation in forensic clinical practice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing how availability and development of SFT could be an effective treatment for severe personality disorders in forensic patients. The analysis includes three years' work with treatment professionals in the Netherlands to adapt and integrate SFT (also known as "Schema Therapy") in their work with forensic patients.

**Introduction:** SFT was developed by Jeffrey Young as a treatment for patients with personality disorders and other difficult-to-treat problems, who often show poor outcomes in other forms of therapy. In a multi-center randomized clinical trial that was completed in the Netherlands, patients with borderline personality disorder who were given SFT showed substantial improvements in their symptoms and functioning over a three-year course of treatment, as well as over subsequent one-year follow-up intervals. It was in this spirit that Bernstein, Arntz, et al. undertook the project of adapting and testing the efficacy of SFT methods in forensic patients with personality disorders.<sup>1</sup> Patients with severe personality disorders, such as those often seen in forensic settings, present special challenges because of their fluctuating emotional states. In the SFT model, these states are conceptualized as "Schema Mode." Recent research suggests that standard cognitive and behavioral approaches are only of limited effectiveness in forensic patients with personality disorders. SFT may provide a more effective alternative for forensic patients with personality disorders.

**Results:** From a SFT perspective, one can understand a forensic psychiatric institution as a complex group dynamic in which the patient's Schema Modes evoked corresponding Schema Modes in various staff members, including the therapist.

Schema Mode Work is the preferred form of SFT practice with more severe personality disorders, such as antisocial, borderline, narcissistic, and paranoid personality disorders, which are the most prevalent personality disorders in forensic settings. It is proposed that the Schema Mode model be expanded to include four new Schema Modes that appear to be common in forensic patients, beyond the 11 original modes that were proposed by Young and colleagues. Schema Modes often play themselves out in a predictable pattern. In some instances, these temporal sequences of unfolding Schema Modes may help to explicate the events leading up to and culminating in the commission of crimes.

From a Schema Mode perspective, it is hypothesized that highly psychopathic patients make prominent use of some of the most maladaptive and destructive Schema Modes; particularly predator, conning, self aggrandizer and bully and attack.

SFT principles and methods have been integrated into each phase of treatment from intake to discharge. The goal of Forensic SFT is to reduce the patient's reliance on maladaptive coping modes by: breaking through the patient's emotional detachment to access and heal their "Vulnerable Child Mode." The findings are preliminary, but they suggest that SFT may be a promising form of treatment for forensic patients with personality disorders. The apparent success of SFT patients in getting permission for leave is an important clinical indication that they are being judged to have a lowered level of risk.

**Conclusion:** This study's adaptation of SFT for forensic patients and recommendations for clinical practice represent a work in progress. They will need to await the findings from their complete sample before making more definitive recommendations regarding triage to SFT for forensic patients with antisocial, borderline, narcissistic and paranoid personality disorders. The ongoing research will provide more insight into the mechanism that explains the apparent effectiveness of SFT in forensic personality disorders patients, including some psychopathic patients. The group is engaging in a board research program to answer questions about the effectiveness of SFT in forensic populations, such as which patients benefit the most from SFT. It is hoped that this study inspires others to investigate other promising forms of therapy for forensic patients with personality disorders. Randomized clinical trials remain the goal standard for investigating the effectiveness of treatment. Without them, there is likely to be little progress in determining what is effective for this highly challenging group of patients.

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#### Forensic Setting, Personality Disorders, Schema Therapy



## I31 Suicide in a Mountainous Territory: A Ten-Year Retrospective Survey

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**Learning Overview:** After attending this presentation, attendees will understand a suicidal trend in the Italian region “Valle d’Aosta” during a ten-year-long retrospective survey.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about unusual aspects of a suicide phenomenon in a mountainous territory that can be a predisposing factor to social isolation and difficulty in health care access.

Italy is ranked 128<sup>th</sup> worldwide for suicide with 4.7 suicides per 100,000 people per year. Since 2013, the leading region for suicidal events in Italy is the “Valle d’Aosta,” a small mountainous area in northwestern of Italy. It is the least densely populated Italian region with only 39 residents per square kilometer. The majority of the population lives in an urban area along the central valley that leads to France and Switzerland, while secondary lateral valleys have only remote rural villages, which are frequently isolated during the long, harsh winters.

This presentation reports data about the total number of suicides in “Valle d’Aosta” from January 2009 to June 2019 collected through the analysis of death certificates and external examination reports archived in the Aosta Office of the Medical Examiner. Resulting data were checked with medical records of the regional hospital to identify cases with a psychiatric history.

The research yielded a total of 159 suicides (20.8% of all the violent deaths): in 43 cases, the decedent was female (27%) and in 116 male (73%) with an average age of 58.6 years (range 17–93 years). The greatest percentage of suicides occurred in the age group of over 65 years (44%). This figure was markedly higher than the national statistics (33%). For this reason, this study compared the older suicidal population (Group 3) with two younger groups (Group 1, aged 17–4 years, and Group 2, aged 35–64).

The male-to-female ratio was 12:1 for Group 1, 1.73:1 for Group 2, and 3.37:1 for Group 3. These data confirm sociological studies: there are distinct sex differences in suicide mortality and suicidality (i.e., ideation and/or attempt). Females report higher rates of attempting suicide, yet males are more likely to die from suicide, possibly because males use more lethal means.<sup>1</sup>

The suicide rate in the lateral valleys was significantly higher in the youngest group compared to the oldest one ( $p=0.0207$ ), probably due to social isolation and lack of a future outlook, more difficult to cope with by a younger population. Seasonality showed a slight homogeneous prevalence of suicides during spring, consistent with the literature on the subject.<sup>2</sup> Leading suicidal methods were hanging (53.8%), fall from height (19.2%), and railway suicide (11.5%), with no firearms cases, for Group 1; hanging (47.6%), fall from height (15.9%), and drowning (14.3%) for Group 2; and hanging (45.7%), fall from height (15.7%), and firearms (15.7%) for Group 3. Hanging is worldwide one of the most common suicidal methods, while the constancy of fall from height as second-most common suicidal method in the present sample can be related to the peculiar morphology of the mountainous territory with cliffs and bridges across waterways.

Among the decedents, psychiatric records were found in 19.2% of Group 1 (mostly bipolar disorder), 34.9% of Group 2 (mostly depression), and 18.6% of Group 3 (slight prevalence of depression over bipolar disorder). The leading method chosen by the psychiatric population of Group 3 was—by far—fall from height (53.8%). This can be explained with the major physical and mental impairment of this subgroup of psychiatric and elder subjects, as fall from height only requires a residual ambulatory ability and minor planning of the act. In all the psychiatric subgroups, no cases of firearms suicides were observed; this is consistent with the strict Italian statutory requirements for firearms licenses. The suicide place was more frequently the decedent’s home for all the groups. While home (70%) outdistanced the other suicidal places in Group 3, such as waterway (8.6%), and bridge (5.7%), in the other groups, the prevalence was slighter. The choice of an intimate place to commit suicide can be related with a higher sense of discretion in the elder population.

This presentation provides attendees with a greater awareness of the importance of social and geographical factors in the forensic reconstruction of the suicidal act.

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### Suicide, Social Isolation, Elderly Population



### I32 Maternal Filicide: A Descriptive and Follow-Up Study of 17 Women Hospitalized in a French Secure Unit Over a 23-Year Period

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**Learning Overview:** The goal of this presentation is to provide an analysis of maternal filicide and to specify the psycho-criminological profile of mentally ill filicidal mothers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the understanding of maternal filicide associated with mental illness.

**Background and Goal:** Maternal filicide is defined as child murder by the mother. Many classification systems have been proposed, based on underlying motives such as altruism, mental pathology (often acute psychosis), an unwanted child, fatal maltreatment, and spousal revenge. Pathological filicide refers to cases in which the perpetrator has a major psychiatric illness. Related literature is sparse, especially concerning the evolution of offenders. The present work seeks to identify the main characteristics of mentally ill filicidal mothers and to assess their socio-clinical evolution after the filicide.

**Method:** Studied first was the sociodemographic, clinical, and forensic characteristics of all filicidal mothers admitted to France's Henri Colin secure unit between 1996 and 2018 (17 patients). The evolution of these patients was assessed after discharge from the secure unit, using questionnaires sent to the prisons or the psychiatric hospitals where the patients were transferred.

**Results:** Most filicidal mothers had a dysfunctional childhood, marked by emotional neglect, intra-familial violence, or social isolation. They were mostly married, with a mean age of 32 years. There was a history of abusing their own children in one-third of the cases. The victims' mean age was 6.2 years, and in several cases, multiple siblings were murdered. Half of the filicidal mothers had a history of psychiatric disorder. Three different diagnoses were found in this study: a mood disorder (41.2%), schizophrenia (41.2%), or a personality disorder (17.6%). The analysis of the act allows us to distinguish two subgroups of motivational profiles. In the first group, filicide is a defensive reaction to a perceived threat: the murder is committed under the influence of hallucinations or delirium. In the second group, filicide is linked to a context of separation, real or imagined. Several forensic and psychopathologic features associated to each profile are detailed. Concerning the evolution of these patients, this study underlines the suicidal risk (even years after the offense), therefore requiring long-term supervision.

**Conclusion:** The results of the present study on 17 mentally ill filicidal mothers are consistent with the literature. Two psychopathologic profiles are described. Several biases in this study disallow the generalization of these findings, and further studies are needed.

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**Filicide, Infanticide, Forensic Psychiatry**



### I33 White-Collar Criminals: A Breed Apart?

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**Learning Overview:** After attending this presentation, attendees will understand: (1) what crimes qualify as “white-collar;” (2) historical models of white-collar criminal behavior; (3) the differential treatment of white-collar criminals from other types of criminals; (4) the evidence basis for the notion of a distinct white-collar criminal; and (5) considerations relevant to evaluating defendants charged with white-collar crimes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) improving understanding of white-collar criminal behavior, and (2) attendees gaining confidence in performing forensic evaluations of white-collar criminals.

The phrase “white-collar” traditionally denotes professionals and others from the upper strata of society. This is reflected in the Federal Judiciary’s use of the term, which first appeared in a Federal decision in the 1930s, referring to a class of workers. In 1947, the United States Supreme Court used the phrase “white-collar” in *Fay vs. New York* to draw a distinction from the “manual laborer.”<sup>1</sup>

Edwin H. Sutherland, the sociologist who is generally credited with coining the phrase “white-collar crime,” defines it as “crime committed by a person of respectability and high social status in the course of his occupation” in his 1949 monograph, *White Collar Crime*.<sup>2</sup> The book highlighted these crimes because, Sutherland observed, they have a great cost to society and were absent from the study of criminology. *White Collar Crime* “ridiculed theories of crime which blamed such factors as poverty, broken homes, and Freudian fixations for illegal behavior.”<sup>3</sup> Sutherland observed that existing theories of criminal behavior were based on biased statistics and specifically neglected “business and professional men.”<sup>2</sup>

Following the publication of Sutherland’s highly influential book, white-collar criminals were no longer ignored. The term “white-collar crime” entered the vernacular. Sutherland’s direct influence on the Federal Judiciary is reflected by the fact that its very first use of the term is a citation of Sutherland’s book in the 1955.<sup>4</sup> Since the publication of *White Collar Crime*, the Federal Judiciary has generally accepted the importance of preventing and prosecuting white-collar crimes.

Ironically, despite Sutherland’s goal of a unified theory of criminality, over time, the idea of the white-collar criminal as a separate class of criminal—a breed apart—has proliferated in the popular imagination. There is the conception of white-collar criminals as typically “ingenious” (so described by the 2<sup>nd</sup> Circuit Court of Appeals in 1961) or even as criminal masterminds (for example, the presentation of Bernard L. Madoff in the media).<sup>5</sup> The idea of white-collar criminals constituting a separate class had become so widely accepted that in 1964, District Court Judge John W. Oliver wrote, “the past sentencing practices of the District Courts of the United States have, I think, not unreasonably produced a longstanding conviction that there is some sort of a distinction between ordinary crime and White Collar crime.”<sup>6</sup> The downside, for defendants, of the idea of white-collar criminals as being a breed apart from “ordinary” criminals is that it may sabotage potential defenses. To wit, a mastermind by definition understands the consequences of his/her actions.

Recently, when examining the 2008 United States financial crisis—an event that uncovered numerous examples of white-collar crime—scholars have rediscovered that such criminal behaviors can be motivated by factors common to other types of crime, including impulsivity, environmental influences, and rationalization. Only time will tell if this conceptual shift foreshadows increased prosecution of white-collar crime or, on the flipside, more successful defenses in terms of mitigation.

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3. Gilbert Geis and Colin Goff. “Introduction,” in *White Collar Crime: The Uncut Version* (New Haven: Yale University Press, 1983), ix.
4. *United States v. Standard Ultramarine & Color Co.*, 137 F. Supp. 167 (S. D. New York 1955).
5. *United States v. Crosby*, 294 F.2d 928 (2<sup>nd</sup> Circuit, 1961).
6. *United States v. National Dairy Prods. Corp.*, 1964 Trade Cas (CCH) P71, 163 (W. D. Missouri 1964).

#### White-Collar, Criminal, Motive





## I34 Pathways to Readmission: Investigating Patient Perspectives in a Forensic Psychiatric Setting

Shannon Kelley, PhD\*, William James College, Newton, MA 02459; Natalie Armstrong Hoskowitz, PhD\*, Bridgewater State Hospital, Bridgewater, MA 02324; Kerri Kane, MA\*, William James College, Newton, MA 02459

**Learning Overview:** The goals of this presentation, in addition to adding to the literature of this understudied population, are to provide a theoretical framework through which readmission issues may be viewed, provide a methodology for other facilities to follow in order to identify patterns to readmission, and present any results from this site that may be applicable and useful to other facilities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a theoretical and practical framework by which to identify and address patterns in forensic hospital readmission rates so that attendees may apply this information in their individual settings.

The average rate of readmission following discharge from a secure forensic psychiatric hospital is high, both in the United States and abroad.<sup>1</sup> This trend is associated with significant financial costs to involved systems, as well as substantial economic, social, and emotional ramifications for readmitted psychiatric patients and their caregivers, such that there is a critical need to better understand and respond to contributing factors. However, the circumstances of readmission to forensic hospitals have received relatively limited scholarly attention, particularly in comparison with the vast body of literature on violence risk. This is concerning given that rates of readmission are much higher than rates of violent offending among discharged forensic psychiatric patients.<sup>2</sup> Furthermore, existing research has largely focused on individual difference variables, and additional investigation is needed to identify potential environmental and systemic factors that may explain pathways to readmission and identify areas for intervention.<sup>3</sup> Prior research suggests that high-risk psychiatric patients are able to prospectively estimate their risk for future violence with a level of accuracy comparable to the leading violence risk assessment instruments.<sup>4</sup> Given that patient self-perceptions are a promising method of improving violence risk assessment, they are likely also a rich source of meaningful information on a broad range of factors relevant to risk of readmission. The purpose of the proposed study is to collect and analyze data from a pilot sample of forensic psychiatric inpatients in order to identify themes regarding the actual and perceived distal and proximal reasons for their readmission to a New England state hospital. The study aims to replicate previous research on individual characteristics of patients at risk for readmission and expand our understanding of the different trajectories and outcomes of forensic inpatients by investigating potential environmental and systemic risk factors.

Data will be obtained from qualitative analysis of in-person interviews of male inpatients from a strict security, New England state hospital. Data collection will likely be ongoing at the time of presentation. Interview questions were designed to capture individual factors that are theoretically and empirically linked to risk for readmission (e.g., substance use, symptom severity), as well as potentially relevant systemic and environmental factors (e.g., access to mental health resources, financial/housing issues, family discord). Data from the subject interviews will be analyzed using one or more qualitative approaches, including a thematic content analysis. This involves reviewing subject responses to define and name common patterns and to identify implicit as well as explicit themes. Results (as available) will be discussed in the context of practical applications for attendees, including how this methodology and any findings may be applicable to other sites for replication or for guidance in how to maximize community reintegration upon discharge. Limitations and future directions also will be presented.

### Reference(s):

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### Readmission, Forensic Hospital, Patient Perspectives

## I35 Shame Among Forensic Patients and the Potential Moderating Roles of the Social Determinants of Health

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**Learning Overview:** After attending this presentation, attendees will have gained insight into the levels of shame among forensic psychiatry patients diagnosed with a psychotic disorder compared to psychiatry patients who are not involved in the forensic system. Additionally, attendees will understand the potential of various social determinants of health in moderating the levels of shame among these populations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing attendees to gain a deeper understanding of the complex social factors that contribute to feelings of shame among forensic psychiatry patients. By acknowledging the prominence of shame, as well as the socioeconomic factors that may contribute to shame, researchers and practitioners within the forensic community will be able to consider certain issues that they encounter with forensic psychiatry patients through a more holistic perspective. Considering shame and socioeconomic factors as part of treatment programs may help reduce levels of shame and, in turn, improve clinical outcomes by allowing patients to advocate for themselves and maintain a sense of hope throughout treatment.<sup>1</sup>

Shame is a painful feeling and experience of believing one is fundamentally flawed and involves a desire to hide the perceived damaged self from others.<sup>2,3</sup> Having a mental health diagnosis can increase the levels of shame a person feels, in part due to stigma surrounding mental illness, and more specifically stigma around psychosis.<sup>4,5</sup> Among individuals with schizophrenia, shame has been associated with negative clinical consequences, including increased paranoia and decreased adherence to treatment.<sup>6,7</sup>

Forensic psychiatry patients comprise a subgroup of individuals with psychotic illnesses who have been less studied with regard to shame. Due to possible further stigma related to contact with the criminal justice system, it was hypothesized that forensic patients diagnosed with a psychotic disorder would have higher levels of shame than patients diagnosed with a psychotic disorder without criminal involvement.

A multitude of socioeconomic factors can impact a person's life and health. These factors are known as social determinants of health, and negative experiences with a number of these factors, such as poverty, have been associated with increased levels of shame.<sup>6,7</sup> It is hypothesized that positive experiences with these factors will lower the levels of shame a patient feels, and negative experiences will increase levels of shame.

This presentation will present findings on levels of shame among patients with schizophrenia and other psychotic disorders, measured using the Test of Self-Conscious Affect (TOSCA-3S). Levels of shame will be compared between patients with a diagnosis of a psychotic disorder who are in the forensic system versus those who are not. Results from moderating analyses will present the impact of the social determinants of health on levels of shame. These results will help clinicians in forensic psychiatry to better tailor treatment programs to their forensic psychiatry patient populations.

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### Shame, Schizophrenia, Social Determinants of Health



### J1 A Fast Examination of Counterfeit Pharmaceutical Packaging Through Laser-Induced Breakdown Spectroscopy (LIBS) and Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) Spectroscopy

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**Learning Overview:** The goal of this presentation is to inform attendees about the advantages, feasibility, and preliminary classification ability of LIBS and ATR/FTIR for the purposes of counterfeit pharmaceutical packaging examination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a fast, portable, non-invasive, sensitive technique for the examination of counterfeit pharmaceutical packaging. In analyzing counterfeit and authentic pharmaceutical packaging, this technique has shown correct classification rates of over 90% using quadratic discriminant analysis for a variety of ink colors and paperboard substrates. These rates indicate that this technique is an encouraging possibility for the analysis of counterfeit pharmaceutical packaging as well as pre-established visual examination methods.

The global production and sale of counterfeit pharmaceuticals present a substantial health and economic risk, affecting thousands of people and costing billions of dollars every year. While covert and overt anti-counterfeit technologies (such as Radio Frequency Identification [RFID], holograms, and security ink) exist to prevent the accurate replication of authentic pharmaceuticals, their cost considerably prevents widespread implementation. As sophisticated counterfeit pharmaceuticals become more difficult to discriminate from their authentic counterparts by simple visual inspection, there is a need for alternative spectrochemical methods for the identification of counterfeit pharmaceuticals and the associated packaging. These spectrochemical methods should ideally have a fast, accurate, and mobile instrument available for rapid on-site detection. LIBS and ATR/FTIR are quick, sensitive, portable techniques to identify the chemical composition of the packaging and packaging ink. LIBS is an inorganic, quasi-non-destructive technique, where only a very small portion of the sample is ablated from the surface, while ATR/FTIR is entirely non-destructive and detects the organic compounds within the sample. Current methods for the examination of suspected counterfeit pharmaceuticals involve the analysis of the Active Pharmaceutical Ingredient (API) and of the pharmaceutical packaging. The investigation of the packaging itself, as opposed to the pharmaceutical, is particularly important in cases of bulk suspected counterfeit pharmaceuticals in which the pharmaceuticals are missing, or when only the packaging has been altered.

In this study, over 150 counterfeit and authentic pharmaceutical packaging samples were analyzed using LIBS and ATR/FTIR. Seven different colors of ink (brown,  $n=21$ ; pink,  $n=45$ ; green,  $n=31$ ; red,  $n=46$ ; yellow,  $n=74$ ; blue,  $n=92$ ; and black,  $n=122$ ) and the paperboard material of the packaging ( $n=147$ ) were analyzed. Each sample was run with eight replicates in different locations to account for variation within the sample; duplicate control samples analyzed on different days were used to assess inter-day and intra-day variations; and samples from the same lot or printed with the same ink/paper/printer system were measured to assess the intra-lot variability. The study included the analysis of packaging from common pharmaceutical products, including hormones, antibiotics, and analgesics. Six machine learning and statistical methods ( $k$ -nearest neighbors, neural network, naïve Bayes, random forest, quadratic discriminant analysis, and linear discriminant analysis) were used to evaluate the methods' capabilities to differentiate between the authentic and counterfeit sample sources and to correctly classify the samples. Fifty percent of the samples were used to train the machine learning classification algorithm; the other half were used to test the classification algorithm. For combined LIBS and ATR/FTIR, the successful classification rate within same-color datasets was over 70% for all techniques and over 90% for the quadratic discriminant analysis technique. These correct classification rates indicate that a combined LIBS and ATR/FTIR technique is auspicious for the non-invasive, rapid (few minutes), on-site examination of pharmaceutical packaging.

#### Counterfeit Pharmaceuticals, LIBS, ATR/FTIR

## J2 Surface Roughness Measurement Techniques Using Pen Pressure Measurement in Signatures and Usability for Determination of Identity

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**Learning Overview:** After attending this presentation, attendees will have better understand the contributions of measuring pen pressure numerically with microscopes measuring surface roughness in determining signature identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the usage of pen pressure measurement, which has an important place in the field of writing and signature examination, and by providing a study on an important subject, the measurement with 3D microscopes.

One of the diagnostic methods used in forensic signature examination and determination of identity is the depth analysis of the stroke and the changes in that value of depth.<sup>1,2</sup> Generally, the methods used to evaluate the depth and depth changes of the stroke do not yield numerical values but rather remain at the level of subjective evaluation.

In this study, the aim is to investigate the use of microscopes that are generally used in the field of engineering for surface roughness measurement and to determine whether the numerical values obtained can play a role in identifications in forensic signature investigations.

The Leica® DVM-6 3D microscope was used to measure the depth of the stroke in this study. Eleven participants were selected, and one was asked to sign their signature three times in three different conditions to create the questioned signatures. A clipboard was used as the first substrate under a blank paper. After that, one paper was placed on the same clipboard as a second condition. Finally, as the third condition, two papers were placed on the clipboard under the blank paper. Five female and five male subjects were asked to imitate the questioned signature three times by only looking at it—freehand simulation. This imitation was performed in all three conditions. Then, three dots were selected from the genuine signatures—those of the person who was imitated them. The maximum depth of the stroke was determined by measuring from these points. The maximum value of the stroke depths was determined for each subject separately by measuring at the same points in the imitation signatures. For each subject, it was investigated whether the values determined from the same points of the signature showed a statistically significant difference with the questioned signature. The data obtained were analyzed in *t*-test using SPSS 23 software. The calculations were performed within a 95% confidence interval.

In the three signatures of the first condition, statistically significant differences were found in six subjects compared to the questioned signature in measurements made from three points (total nine points). In the three signatures from the second condition, statistically significant differences were found in three subjects compared to the questioned signature in measurements made from three points (total nine points). In the three signatures from the third condition, statistically significant differences were found in four subjects compared to the questioned signature in measurements made from three points (total nine points). The mean of all three conditions (mean of 27 points in total) shows statistically significant differences in seven subjects compared to the questioned signature.

It is never possible to reach definitive conclusions in determining the identity of signatures. As matter of fact, when experts write the results, they never say that this signature is the product of a particular person's hand. One of the criteria used in this determination is the depth of the strokes that make up the signature. In addition, it is important to show this depth numerically with the changes created by depth. In practice, experts will never be able to decide on the identity of a signature with the values they achieve. However, they will have an important criterion in establishing an opinion. Herein, it is necessary to evaluate the values obtained, together with other findings, and to interpret them correctly.

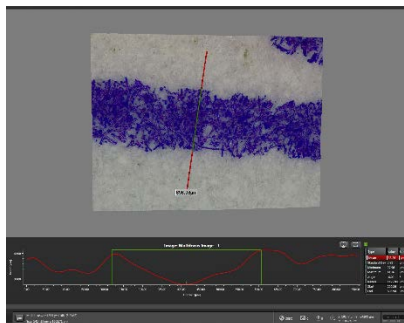


Figure 1: Pen pressure depth analysis sample result that was used in study.

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### Depth Analysis in Stroke, Pen Pressure, 3D Microscope

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### J3 Use of Raman Spectroscopy in Forensic Sciences to Authenticate Artworks Seized by Operation Lava Jato

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**Learning Overview:** The goal of this presentation is to bring awareness to attendees regarding investigating a money laundering and bribery scheme involving numerous public figures, and state and private companies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that samples that present luminescence are currently being discarded as an evaluation method. This study proposes that through the luminescence presented in the Raman spectra, it is possible to differentiate works of art.

As has been widely reported by national and international media, Operation Lava Jato is a set of investigations underway by the Brazilian Federal Police, aimed at investigating a money laundering and bribery scheme involving numerous public figures as well as state and private companies.

During the operations conducted, the number of works that were seized in the past four years were greatly highlighted. By 2016, around 230 works of art had been seized, indicating that the use of works of art has been recurring as a form of money laundering. The choice of works of art in this scheme may be due to the value given to a certain work, the value that can be obtained in an auction, and the lack of technical criteria to say if these works are genuine or not, because the sales market is not regulated as it should be.

After the large number of apprehensions, there was the need to look for techniques in which it was possible to analyze the authenticity of the works. In the past, this work was performed by art specialists, who through visual analysis determined whether a work was authentic or not; but the Federal Police realized that just a visual analysis was not enough, and that it was necessary to use a method that would not be challenged in the future and that would maintain the integrity of the work in question. The Technical Scientific Sector of the Federal Police Superintendence Paraná, headquartered in Curitiba, sought qualified people from universities in optical techniques, preferably non-destructive, such as the Raman spectroscopy technique. A partnership was then made between the Technical Sector of the Federal Police (STPF) and two universities: the University of São Paulo (USP) and the Federal University of Paraná (UFPR).

The work in question was performed by the Federal Police's Scientific Technical Sector with the UFPR, and dealt with the analysis performed on four works of art that have been questioned by the Federal Police regarding their authenticity with a possible signature of the painter and diplomat, Sergio Telles. These works, called Questioned Frames 34, 35, 36, and 113, are under the custody of the Oscar Niemeyer museum, also located in the city of Curitiba in Paraná.

Samples were collected by scraping using a 30G ½ needle. The Raman scattering experiments were performed using a Raman Witec Alpha 300 confocal microscope with an excitation laser operating at 532nm and focused by a 50x lens.

Analyses of the various samples were performed, visually searching for similarities between the pigments of the four frames. The presence of the pigments was identified as: blue, white, orange, brown, and green in both frames; there were cases of a frame having more colors than those mentioned, which were not discarded, because there is future intention of creating a database of national pigments. After determining the colors present, the analysis was performed by color similarity.

In some cases, the Raman spectrum was clearly visible, but in most of the spectra, the luminescence was present, so by analyzing the line shape and the light emission peaks, this study could find other components present in the paintings, as in the case of yellow, orange, red, and green contributions in the samples.

In cases in which the spectrum was clearly visible, it was possible to identify the similarity to the violet diaxazine pigment, which was present in several of the samples. The oranges and browns in two of the works show a great chance of compatibility, in addition to the white present in three paintings, which presented a luminescence characteristic already expected to be this pigment.

From the analysis performed, it is possible to state that there are great similarities between the pictures. The next step to be taken is to perform measurements related to the standard frame, certified by Sérgio Telles himself, compare these with the results already obtained by the questioned frames, and use the Raman technique with infrared excitation laser to decrease the effect of luminescence.

#### Authentication, Artworks, Operation Lava Jato



### J4 The Authenticity of Questioned Pretty Good Privacy (PGP) -Signed Digital Documents

Martin S. Olivier, PhD\*, University of Pretoria, Pretoria, GP 0028, SOUTH AFRICA

**Learning Overview:** The goal of this presentation is to question the authentication of digitally signed documents and whether they will stand the test of time based on a case study involving 55 PGP-signed documents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the many problems encountered (primarily obtaining a public key or finding grounds for trusting a public key). This presentation also points toward basing trust in keys on known good exchanges where the key was used.

In principle, authentication of digitally signed documents is a simple computational process. PGP was created in 1991 as a cryptographic tool that enabled users to communicate privately, but also to achieve a range of other functions, including the ability to sign documents.<sup>1</sup> For over three decades, PGP (or PGP-compatible software) was a standard tool for users who wanted to encrypt and/or sign documents. The open source implementation (the GNU Privacy Guard [GnuPG]) is based on the OpenPGP standard.<sup>2</sup> This presentation uses the term PGP to refer to any implementation PGP of functionality.

While other (better) solutions than PGP have been developed, none have achieved the widespread acceptance and name recognition PGP has. The shortcomings of PGP are well known, but when used correctly, PGP remains useful.<sup>3</sup>

One question about authentication of digitally signed documents is whether they will stand the test of time. This presentation presents a case study in which 65 documents signed with PGP have been examined to determine whether their authenticity can (still) be established. The dates of these documents range from July 22, 1998, to May 15, 2018. The primary tool used for signature examination was Gpg4win—an implementation of GnuPG for Microsoft® Windows®. Gpg4win version 3.1.10 released on July 14, 2019, was used.

The 65 documents were not selected randomly; thus results cannot be generalized. Moreover, problems encountered can be seen as predictable, based on PGP critiques. However, an empirical case study provides insight into the relative prevalence of such expected problems.

The selection of older documents was intentional: The forensic document examiner is often confronted by authenticity of older documents, such as a last will or an old contract. The first problem (predictably) stemmed from the age: the signatures were associated with defunct email addresses.

Consider a specific example: one of the files examined was signed by the signator on October 19, 1998. The key remains available on public key servers, even though the associated address has not been used for well over 15 years. The key is not signed by a third party; the signator removed the key long ago from his keyrings. Backups were found, but were corrupted. Two options existed (claim ownership or sign the key). However, both rely on the signator's recollection of key authenticity. Both lead to "successful" verification of the document.

Some general remarks can be made about observations in this case study. About half of the keys used to sign documents were available from key servers. However, none of the keys were signed by third parties. Hence, key servers were not useful to find a chain of certificates between any key used and the a signator's key. Availability of about half of the public keys made it possible to proceed with verification if grounds existed to trust the public key.

As noted, many keys are linked to obsolete email addresses, which complicates signer identification. Users arguably do not have much information available. In any case, relying on the user's cooperation enables repudiation. Web searches were performed for key fingerprints but yielded no useful information for any search.

Another problem: about half the keys found on key servers had expired. Gpg4win in such a case reports "The signature is invalid: Signing certificate is expired." GnuPG 1.4.20 and GnuPG 2.1.11 on Linux® produced potentially more useful results (with the caveat about trusting the key). It reports "Good signature", but warns the user (twice) that the key had expired.

The problem of key authenticity is pervasive. One promising possibility remains: where the key has been used in other exchanges known to be valid, such knowledge may be used as grounds to trust the key. An email interchange between two users who know one another is one possible example.

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3. Whitten, Alma, and J.D. Tygar. 1999, August. Why Johnny Can't Encrypt: A Usability Evaluation of PGP 5.0. *Proceedings of the 8<sup>th</sup> USENIX Security Symposium*. Washington, DC, 169–184.

#### Digital Signatures, Questioned Digital Documents, PGP



### J5 What's Old Is New Again: Portable Instant Photo Systems and Questioned Documents Redux

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**Learning Overview:** After attending this presentation, attendees will have a more thorough understanding of currently available instant photo technologies and features associated with those prints.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) increasing awareness of instant photo technology; (2) offering insight into photo production processes; and (3) illustrating how these prints could be identified in questioned documents.

Invented in the 1940s by Edwin Land, Polaroid® dominated the instant film market for decades, up until it was uprooted by 35mm film and emerging digital formats in the 1990s. After an extended lull in popularity, the incorporation of digital photography with modernized printing technologies led to a resurgence of an updated form of the once-considered vintage instant film system. Although Land's invention has had staying power in today's society, the reality remains that the newer generation of document examiners may have limited experience with instant photography systems. As the potential for improved technology in this area continues to evolve, there is a need for this knowledge gap to be addressed in order to ensure thorough examination of identification documents.

Documents associated with identity often include a photograph. The properties of these documents are dependent on a number of factors, including the issuing country, the requirements at the time of issuance, and the document's purpose for use. Exposure to a broad range of document types has increased due to the breadth and accessibility of international travel and migration, which has in turn led to the necessity of examining all types of unfamiliar identity documents. In the event that a document becomes questioned, identification of production technologies is often required. To properly analyze the value of any findings, awareness of currently available print process technologies, including instant films and their associated features, should be taken into account.

The availability of these low-cost and high-quality instant film systems provides examiners with another factor for consideration as to how a document might have been constructed. Traditional instant film development was based on silver halide exposure with reagent development, producing the expected low-quality instant print. The more current instant photo systems utilize digital photography with integrated reagent development film, or form factor thermographic print processes producing higher-quality images. As a result, more regard should be given to alternate sources of photograph production.

The scope of this presentation will be limited to technology suitable for portrait photo production, as is typically encountered in identity documentation. A non-exhaustive selection of form factor printer devices will also be used, limited to popularity of use. This presentation will provide attendees with an overview of currently available portable instant print photo systems, including portable instant printers, the properties associated with these various technology types, and features that could be used to aid in identifying these products.

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#### **Questioned Documents, Instant Photography, Print Process**



### J6 Delving Into Digitally Processed Documents: How Does Optical Character Recognition (OCR) Impact Documents?

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**Learning Overview:** After attending this presentation, attendees will understand the impact of OCR technology on various documents containing signatures, writing, different writing instruments, and text typed on a typewriter.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on how OCR technology may impact a document that is being examined.

OCR or optical character reader is the process of converting images containing text into machine-encoded text. The images can be scanned or photographed, and a software application converts all text components. OCR integrates three research fields: pattern recognition, artificial intelligence, and computer vision.

In the digital age of artificial intelligence and automation, OCR has become popular for various applications. Documents containing text that have undergone OCR processing can be electronically searched, edited, stored, and uploaded. In addition, this digitized text can be used in machine processes, such as cognitive computing, text-to-speech, machine translation, and text mining.

Forensic Document Examiners (FDEs) may be asked to examine digitally processed documents. Some businesses are shifting toward becoming paperless. This shift within some businesses has resulted in FDEs examining more digitally processed documents. With the aid of technology, four individuals from four corners of the globe can all sign one document without ever having to sit around the same table. Signatures from four different individuals would require digital processing of the document, likely scanning and re-scanning of the document. What does this document look like after undergoing multiple digital processing steps?

This research sets out to determine how OCR technology impacts documents with text. There are numerous OCR software applications available. The documents in this research project were processed with the OCR function in Adobe® Acrobat® Pro DC. How does Adobe® Acrobat® Pro DC OCR read different signature styles? How are these same styles of signatures reproduced when scanned and converted into a text document using the Adobe® Acrobat® Pro DC?

Documents can be written using various writing instruments. Is there any difference in how the Adobe® Acrobat® Pro DC OCR function reads and converts the text for different writing instruments?

Some documents contain a combination of typed and written text. How does Adobe® Acrobat® Pro DC OCR read cursive or printed or mixed (both cursive and print) text? How are these same writing styles reproduced when scanned and converted into a text document using the Adobe® Acrobat® Pro DC?

One may want to digitize their old records, including documents that have been composed on a typewriter. How does Adobe® Acrobat® Pro DC OCR read typewritten documents? How are typewritten documents reproduced when scanned and converted into a text document using the Adobe® Acrobat® Pro DC?

This research will also examine some of the mobile OCR applications, including Scanbot®, Dropbox®, Adobe® Scan, Microsoft® Office Lens, and Google® Keep.

In this digital age, FDEs should be cognizant of how digital processing techniques such as OCR may impact documents. Artifacts and digitization can be observed in digitally processed images. Understanding how the artifacts or digitization occur is important. If no original document is available, the best available copy should be requested. If only a digital file is available, the digital file in its original file format should be submitted for examination. An FDE may be asked to examine a hard copy of a document that has undergone multiple digital processing. It is important that FDEs understand the evidence that digital processing applications, such as OCR, may leave behind on the document and how the digitally processed document may differ from the original or even the first-generation copy.

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#### Optical Character Recognition (OCR), Document Processing, Scanned Document





## Questioned Documents—2020

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### J7 Application of the “Value of Evidence” Approach in Forensic Document Examination

*Miriam Angel, MS\*, Los Angeles Police Department, Questioned Documents, Los Angeles, CA 90032*

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**Learning Overview:** The goal of this presentation is for attendees to acquire a working knowledge of how the “value of evidence” approach can be applied to document casework through illustration of theory basics and mock case examples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing modifications to the current interpretation method and reporting conventions that can help elucidate the evaluation process and advance forensic document examination as a science.

Numerous publications in the scientific, academic, and legal communities have described the need for forensic conclusions to be scientifically justified, with an aim toward balance and transparency. The widely suggested method for evaluating and reporting forensic findings requires that the examiner consider the value of evidence in terms of the likelihood of observing the evidence given at least two competing propositions, within the framework of the case information. This is opposed to expressing opinions on the likelihood of the propositions themselves. It is advisable that examiners be familiar with the logic behind the different types of conclusion verbiage so they can speak intelligently when this topic inevitably finds its way into the courtroom.

This presentation will involve discussion of the theory behind the “value of evidence” approach and how evaluation under this method would differ from the traditional method of evaluating document evidence. Sometimes referred to as the “logical approach,” interpreting evidence and expressing conclusions in terms of the “value of evidence” means the examiner considers the likelihood of the evidence given each of a pair of propositions and then weighs the difference in the magnitude of support for each of those propositions. By explicitly considering each proposition, the examiner makes clear that each side of the dispute was given due attention as well as how much support was qualitatively assigned to each.

Using a mock case, attendees will be guided through each step of the examination process, from stating the relevant propositions to outlining the expectations of observations under each proposition to evaluating the likelihood of the evidence given each proposition, and then, finally, to expressing an opinion. Assigning individual likelihoods and consolidating those likelihoods into a logically based conclusion statement will be discussed. How those assignments can be justified will also be advised. In this presentation, the “value of evidence” conclusion will be given in qualitative terms rather than numerical ones.

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#### **Evaluative Reporting, Conclusion, Document Examination**



### J8 Characteristics Observed in Impressions Produced by Signature Stamps

*Jan Seaman Kelly, BA\*, Forensic Dynamics LLC, Las Vegas, NV 89147*

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**Learning Overview:** After attending this presentation, attendees will be aware of the various materials used for stamp dies and the characteristics observed in the impression of a signature stamp.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating attendees of the materials used for the dies of facsimile signature stamps. Having an understanding of the characteristics observed in an impression may assist the examiner during the examination in determining the type(s) of rubber stamp(s) that could have produced the signature stamp impression.

The definition of signature has changed as it was limited to a handwritten name or mark made by the individual onto a document as proof of identity and intent. The legal requirement that a signature had to be handwritten was changed when facsimile signature stamps began to be used in business. With the development of the computer, electronic and digital signatures are heavily relied upon to complete business transactions. The impact of digital technology has impacted the manner in which individuals sign their signature on personal and business documents. Even though this has impacted the number of facsimile signature stamps being used, the forensic document examiner may encounter a case involving a facsimile signature stamp as this remains a viable option to sign one's name to a document.

Facsimile signature stamps are used to sign a myriad of documents, including business or personal letters, checks, invoices, legal documents, certificates, etc. Signature stamps are easy to use and guarantee a perfect impression of the signature every time. The use of a facsimile stamp signature on a document implies the same intent and legality as if it was an original inked signature manually produced by the individual. The advantage of using a facsimile stamp instead of manually signing the signature is one of convenience. The disadvantage of using facsimile stamps is the stamp can be stolen, duplicated, and used for criminal activities.

The examination of a signature stamp impression or a facsimile signature stamp is within the scope of forensic document examination. The dies of facsimile signature stamps include laserable rubber, photopolymer, and flat die. Water-based stamp ink is primarily used for rubber and photopolymer dies while flat die stamps use oil-based ink. Characteristics observed in the signature stamp impression can be clues as to the type of die used to produce the impression. For example, laserable rubber and photopolymer dies are raised and can leave an outline or "squeegee" appearance on the edge of each letter in the signature while the flat die stamp will not leave an outline.

In modern business, one acceptable method of signing a document is the facsimile signature stamp. This presentation will discuss the types of die materials used and the related characteristics and defects that can be present in the stamp signature impression.

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**Forensic Document Examination, Signature Stamp, Stamp Die Material**



### J9 Testing the Perceptual Accuracy of a Subject's Ability to Identify Their Own Handwritten Numbers and Words

*Jacqueline Joseph\*, Portland, OR 97205*

**Learning Overview:** After attending this presentation, attendees will understand that some people cannot recognize their own handwriting and handwritten numbers. This presentation will illustrate an original research method that acquires data for use in overcoming a general assumption that people can successfully recognize their own handwriting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering insights about a person's subjective statement of "That's not my handwriting" or "It looks like my handwriting, but I don't remember writing it" by applying forensic handwriting identification methodology to show evidence that it is their handwriting. This presentation will illuminate the issue of eyewitness error in which a subject is unable to identify their handwritten words or numbers in records and other documents.

This presentation will illuminate the issue of eyewitness error when a subject denies, is unable to, or mistakenly identifies his/her handwritten words and/or numbers in cases involving: (1) altered medical records; (2) employment logs/timesheets; (3) legal documents; and (4) other questionable handwritten entries. Knowing these insights may become the basis of serving the truth when a subject cannot recall or is unable to accurately recognize and successfully identify the questioned handwritten numbers and/or words.

Warned of the unreliability of eyewitness testimony, someone may be confounded when comparing the statement of his suspect, "That's not my handwriting" with the proffered expert opinion of "The suspect wrote it," or vice-versa. Outside the specialty of Forensic Document Examination (FDE), some people believe that they can successfully identify their own handwriting.

Prompted by credibility challenges of expert handwriting opinion, a small population study with 23 subjects was designed and conducted for the purpose of securing data on the success of subjects and their ability to recognize their handwriting and handwritten numerals.

Handwritten numbers are written similarly in various languages; therefore, they cross borders, allowing subjects of many nationalities to participate in the study. Additionally, the following confidence choices were included: "I'm 100% certain," "I'm fairly certain," "I'm guessing/I don't know," and "My writing is not observed on this exhibit."

In summary, the results of this study revealed that 98% of the subjects successfully identified their handwriting, while 50% of the subjects failed to successfully identify their handwritten numbers. The handwritten numbers used for this study included: "April 15, 2007," "1948," "78910."

In conclusion, because the Forensic Document Examiner's expert opinion is tested for reliability based on the sufficiency of evidence coupled with the examiner's skill, knowledge, and experience, the awareness of the results of this study may become an important factor for use as the basis for forming a reliable opinion.

There is much to be gleaned from this study. For example, statistics regarding the subjects' confidence choices may be tabulated for an expanded version of this study. Additionally, it would be insightful to acquire the subjects' confidence choices before the study's purpose was revealed, and prior to the handwriting of the phrases and numbers. Furthermore, subjects' bias or intentional disguise could factor into the overall dilemma of conflicting opinions on the same evidence.

The preliminary data from this small population study suggests additional research would be fruitful.

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#### **Eyewitness Error, Opinion Conflict, Handwriting Perception**





### J11 Education and Training in Forensic Document Analysis Offered as an Elective Course to Undergraduate Forensic Science Students in Turkey

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**Learning Overview:** After attending this presentation, attendees will understand how an elective course in the field of forensic document analysis has been offered at Istanbul's Uskudar University to undergraduate forensic science students by academicians with a professional expertise in the field for more than 20 years.

**Impact on the Forensic Science Community:** This presentation will impact the forensic sciences community by presenting details about the one and only teaching and training program about document analysis at the undergraduate level in Turkey.

In Turkey, as with every developed country, it is necessary to instruct any interested candidate well equipped with knowledge and experience in forensic science at the undergraduate level to prepare them for conducting scientific research, for developing novel methods and instruments, or for future positions, such as private or government crime laboratories. Transforming expert establishments, whether private or governmental, and crime laboratories into organizations that are accredited and well-known in Europe and around the world, and enabling the export of high technology heavily depends on the aforementioned. The Forensic Science program under Uskudar University's Faculty of Engineering and Natural Sciences is the first and still the only one in Turkey, offering BSFS degrees. The learning content was prepared in accordance with the Forensic Science Education Programs Accreditation Commission (FEPAC). FEPAC is a body of the American Academy of Forensic Sciences (AAFS), founded in 1948, which currently has more than 6,500 members and is the only association in the world that accredits undergraduate and graduate education.

Efforts to strengthen the reliability of the methods of forensic practitioners have led to attempts to create standardized teaching and training programs, especially in the field of pattern recognition; document analysis and signature identification is one such field. An elective course is offered in the fifth and sixth semester to teach the basics of this field and train the students accordingly. The course does not train the student as a document examiner and in no way certifies or qualifies the student to conduct questioned document analysis for the court. The syllabus encompasses: (1) the functions and responsibilities of questioned document examiners in the criminal justice system; (2) the process for obtaining exemplars, types of document examination (e.g., handwriting, typewriting, inks, erasures), collection and preservation of evidence as well as courtroom procedures; (3) the theoretical and practical aspects of handwriting as forensic evidence; and (4) the production of normal and false handwriting, variables in handwriting production, standards of comparison, identification theories, examination methodologies, expression of conclusions, and challenges to professional practice. Students have hands-on training according to the best practice manuals, standards, and guidelines published by the Scientific Working Group for Forensic Document Examination (SWGDOC), the American Society for Testing and Materials (ASTM) E1732, and the European Network of Forensic Science Institutes (ENFSI) and will learn the operation and practical use of various instrumentation used in the field, such as the Video Spectral Comparator (VSC) 8000 and Leica® Z6 Stereo Macroscope and Leica® FSC Comparison Microscope.

#### Document Analysis, Signature Identification, Forensic Science Education



### J12 Investigating the Interlaboratory Reproducibility of Magnetic Flux Measurements of Toners

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**Learning Overview:** After attending this presentation, attendees will better understand the potential for use of a quantitative magnetic flux measuring device to differentiate between black and white toner-printed documents from different sources. Attendees will gain an understanding of the magnetic characteristics exhibited by toner-printed documents and how to employ magnetic flux measurement techniques during comparative examinations between questioned and reference printed texts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a method for rapidly screening black and white toner-printed documents, offering the opportunity to evaluate the subsequent need for more time-consuming or destructive methods.

This study was conducted to address questions raised by previous research into the magnetic properties of toner and the use of magnetic flux measurement devices. Specifically, this research addresses questions related to the reproducibility of numeric magnetic flux measurement results between different analysts from different laboratories using similar instrumentation. The hypotheses advanced were that the difference in magnetic flux measurement values produced by different devices is not significant, or alternatively significant, when applied to the same sample.

To test the hypothesis of interest, samples were prepared that contained a standardized test site with a grid of squares of known area and controlled properties to minimize the chance of hysteresis and other induction current spatial effects. The study sample set consisted of three toner samples for each participating laboratory as well as a standardized negative quality control sample. These samples were analyzed by the issuing laboratory and three participating laboratories using a standardized methodology guide optimized to control for hysteresis and to minimize operator-introduced random error. Following this methodology guide, each laboratory conducted testing on positive and negative quality control samples at instrument startup before collecting 28 replicate measurements on each toner sample. The toner samples were measured by the originating laboratory prior to issuance to the participating labs to facilitate the direct comparison with the results of the participating laboratories. The values obtained for each toner sample by each participating lab were then compared with the values obtained for that same sample by the issuing lab via a paired *t*-test to check for significant variation in the results before and after performing comparisons between the results of all of the laboratories and using Analysis Of Variance (ANOVA).

It was found that there was a significant difference in magnetic flux measurement capability between similar devices, and the values produced for the same sample were significantly different. Some of the participating laboratories were not initially able to follow the standardized methodology guide when it was presented, as the software and sensor versions for their instruments were significantly different and not originally set to conduct the requested measurements. These investigations have found differences in the instrument serial number to be a significant source of variability in magnetic flux measurement data. For effective implementation of magnetic flux measurements in forensic laboratory settings, it is advisable to ensure that measurements are conducted using the same device when their numeric results will be the basis for comparisons. Numeric measurements of the magnetic flux of toners can be of value for questioned document examiners due to their non-destructive nature and their rapid collection; however, it is important to ensure that the methodology used controls for sources of variation, including differences in the device and software design.

#### Questioned Documents, Toner, Magnetic Flux



### J13 Decipherment of Latent Handwriting Impressions: Point/Counterpoint

Thomas W. Vastrick, BS\*, Apopka, FL 32703

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**Learning Overview:** After attending this presentation, attendees will understand what should be expected of a forensic document examiner concerning the decipherment of images raised through an Electrostatic Detection Device (EDD) or special lighting, what they should be able to do, and what limits they should impose, if any.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing a consensus methodology for use in everyday casework involving the decipherment of latent handwritten images so that the resulting conclusions will be more reliable and more uniform. In addition, accuracy will be increased by a consensus understanding of the limitations imposed on such procedures.

Forensic document examiners commonly, but not exclusively, conduct examinations of latent handwriting impressions utilizing an EDD. This device is the best practice for examinations in which use of the device is possible. Lighting from various angles are also used as an enhancement to the use of EDD and sometimes, by various necessities, as a substitute. Either method is applicable to the theme of this presentation.

The Scientific Working Group for Forensic Document Examination (SWGDOC) is an organization dedicated to developing and publishing discipline-wide standards in forensic document examination. One such standard is titled *SWGDOC Standard for Indentation Examinations*. In this standard are statements as to decipherment of indentations of latent handwriting impressions in Section 8.3 as follows: 8.3.—once examinations and evaluations have been completed, reports may include the following types of conclusion(s), opinion(s), or finding(s): 8.3.3.—the text of deciphered indentations.

A question arises as to: (1) whether a forensic document examiner is trained to decipher handwriting; (2) whether the experience of a forensic document examiner qualifies one to decipher writing; (3) whether there are any proficiency studies to establish that a forensic document examiner has a superior ability through either training or experience to decipher handwriting; and (4) whether there are limitations that should be placed on this process. It is the purpose of this presentation to address these specific four issues and initiate a debate concerning any needed modifications to the published standards if it is deemed necessary.

Attendees will be exposed to various opinions and ideas as to decipherment. It is the purpose of this presentation to present both sides objectively and moderate a discussion with attendees. As a result, attendees should come away from this presentation with a better understanding of the foundational bases for stating conclusions or limiting conclusion statements in regard to decipherment of latent handwriting impressions.

One position is that forensic document examiners are trained in the decipherment of latent handwriting impressions. In the standard titled *SWGDOC Standard for Scope of Work of Forensic Document Examiners*, Paragraph 4.2.2 (concerning forms of examinations) states: Other problems are the decipherment and sometimes the restoration, alteration or additions to document and relationships of documents. Some of the considerations as to a separate expertise in decipherment involve: (1) the specific training in decipherment; (2) the impact of the examination of large quantities of writing as a foundational basis for decipherment; (3) study of indentation obstructions and their impact in impressions; and (4) study of various forms of writing to include date formats, time formats, diacritics, etc.

Since this research is unaware of any proficiency studies as to decipherment capabilities of professional forensic document examiners versus a control group, a discussion of the design of research and a search for willing researchers will be a part of the general discussion. Next, there will be a short review of various form variations, such as date entries, phone numbers, time, etc. The purpose of this section is as a refresher to understanding that alternative forms must be considered in any decipherment process. Finally, this discussion will seek to reach a consensus as to report terminology that accurately reflects both the findings and the limitations to the noted examination data.

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#### Handwriting, Indentations, Decipherment



### J14 Infrared (IR) Luminescence With Different Paper Substrates

*Dennis J. Ryan, MBA\*, Applied Forensics LLC, East Meadow, NY 11554*

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**Learning Overview:** The goal of this presentation is to determine if different paper substrates have any effect on the visualization of luminescent pen inks.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how an examiner must be cautious in drawing any conclusions when different paper substrates are involved.

The paper substrate variables included brightness, weight, brand, and type of paper. The preliminary research determined that there was a significant variability within one level of brightness (92, 98, 102, etc.), so the research was predominately restricted to only 98 brightness paper. Eighteen different pens were used with numerous different paper substrates. Individual pieces of 98 brightness paper was used. These pieces varied by weight, type, and brand. The size of the piece of paper remained constant. One straight ink line was drawn with 18 different pens of varying colors and types. Each pen used was known to have ink with luminescent properties. The different pens were broken down into 3 rollerballs, 2 fountain pens, 1 felt tip, 11 ballpoints, and 2 gel pens. This study used the following 98 brightness papers: four color copy papers, seven multipurpose papers, six laser papers, and one inkjet paper. A Foster & Freeman Video Spectral Comparator (VSC) 40/HD was used to detect the IR luminescent properties of the different pens. Eighteen pens were used, but data was analyzed for only 17 pens; one of the pens ran out of ink. Significant variations were seen when comparing multipurpose to color copy paper.

Caution should be exercised when examining the IR luminescent properties of inks over different substrates. The findings showed that the type of paper may have a significant effect on the visualization of luminescent inks. Inks luminesced at lower wavelengths on laser and color copy papers than the same ink on multipurpose papers. No correlation could be made between paper weight and ink luminescence because no consistent pattern was observed. No one brand of paper consistently affected the visualization of the luminescence. Erroneous conclusions may be reached when examining inks over different paper substrates (i.e., unlined white paper enclosed in a white envelope).

Some preliminary results were obtained from 96 brightness white paper, 100 brightness, and color copy paper. The results from the 96 brightness paper was consistent, if not identical, to the results found to the same type and weight. Some pens luminesced under slightly lower wavelengths on laser and color copy paper on the 96 brightness paper. The results for the 100 brightness papers was less conclusive because there were as many different paper substrates on the commercial market as there is in the 96 and 98 brightness range.

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#### IR Luminescence, Ink Examination, Paper Substrate





### J15 X-Ray Cabinets Applied to Forensic Document Examination

*Samiah Ibrahim, BSc\*, Ottawa, ON K1S 1R1, CANADA; Tobin A. Tanaka, BS\*, Canada Border Services Agency, Ottawa, ON K2E 7M6, CANADA*

**Learning Overview:** After attending this presentation, attendees will learn how X-ray cabinet radiography can be used in examinations of questioned documents and the materials used to produce documents.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by demonstrating how X-ray cabinets can be used in a variety of examinations in the questioned document laboratory.

Questioned document examination is one of the oldest areas of forensic science, spanning more than 100 years, coincidentally emerging around the same time frame as when X-rays were first reported in the scientific literature by W.C. Röntgen.<sup>1</sup> Documents may take many forms, for example: single sheets of paper, bound books, packaging, and plastic cards. The increase in the different types of materials, such as ink, toner, papers, and polymers that may be used in making documents, is also diverse. Consequentially, the range of instrumental techniques and technologies used in their examination is equally diverse.

X-rays are commonly used in non-destructive examinations of objects in industry, medicine, dentistry, and materials science. It follows that its use in forensic science will apply to many situations where similar types of non-destructive examinations may be beneficial, such as those encountered in questioned documents. Forensic document examination has used radiography and in particular “soft” X-rays to reveal the inner structures of documents and the materials used to make them. While specific application of X-rays to document examination has been published at least as early as 1953, it has not always been straightforward to obtain suitable radiographs.<sup>2</sup> The difficulties are not always solely due to the time taken for film processing but to the fact that most X-ray equipment is not well suited for low-density materials, such as documents. Many instruments are not capable of producing X-rays of low enough energy to disclose these subtle radiographic densities. As a result, many X-ray devices and sources are too powerful, resulting in poor contrast radiographs. Other challenges include requiring specialized knowledge to operate certain devices and shielded areas for health and safety regulatory requirements.

The characteristics of X-ray exposure and nomenclature will be reviewed. This will educate the document examination community on how radiograph exposures are described in terms of factors such as: X-ray tube voltages and current, exposure time, beam diameter, sensor capability, and geometric magnification. Some basic physics on X-ray interactions with matter will provide insight into what happens when an object is subjected to X-ray radiation. These interactions include scattering, transmission, and absorption, and these may pose radiograph imaging problems, especially when the shape of objects is irregular.

Many practical lessons on successful implementation of X-ray radiography of documents can be drawn upon from the conservation and restoration community. Objects including documents and books are subjected to X-ray radiography in a similar fashion to what may be desired for matters pertaining to forensic document examination.<sup>3</sup>

Modern cabinet X-ray devices afford the convenience of instant results, flexibility in setup, and safety and convenience of digital radiographs, permitting adaptable post-processing for optimal viewing. This presentation will include several examples of different document types along with discussion on how this type of non-destructive examination can provide valuable insights into document construction, alterations, and materials differences. Digital radiograph formats including the Digital Imaging and Communications in Medicine (DICOM) will be explained as will the advantages and disadvantages of each.

#### Reference(s):

1. W.C. Röntgen. On a new kind of rays. *Nature*, Vol 53:274-76.
2. Harris B. Tuttle. X-Rays Held to Trip Stamp Counterfeiters. *Identification News*, November 1953, p5.
3. Janet Lang and Andrew Middleton (Editors). *Radiography of Cultural Material*. Elsevier Butterworth Heinemann, 2005.

#### Questioned Documents, X-Rays, Radiography



## Questioned Documents—2020

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### J16 Practice Management Software: The Key to Staying Organized

*Linda L. Mitchell, BS\*, Forensic QDE Lab, LLC, Escondido, CA 92025-4116*

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**Learning Overview:** The goal of this presentation is to introduce the concept of practice management software and illustrate the advantages of keeping communications, evidence copies, examination records, and billing information in one place.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a user-friendly method of evidence management to keeping records clean and organized. This presentation is directed toward experts of all types in private practice.

Experts in forensics find themselves in an age where documentation and data storage methods are crucial to their credibility. No longer can one put a file on the hard drive for later retrieval without first making it easily retrievable. Important work product and related documents are sometimes required at a moment's notice, not to mention that the file system of one's personal hard drive poses many challenges when trying to locate important information.

In the attorneys' world, a great number of software programs have been developed to help keep evidence, case law, and work time sorted out. Over the years, it has become more inclusive of communications, work product, and time tracking, allowing the attorney to keep all the documents related to a case sortable and searchable. These developments have inadvertently served adjunct legal professionals, such as expert witnesses.

Experts have the same challenges as attorneys, particularly those who are engaged in private practice. While not as closely monitored as certified document labs, private practitioners should be providing a similar regimen of data maintenance and retrieval. Further, there should also be a secure means of transmitting work product and any other data to clients rather than using Gmail® or Yahoo®.

Practice Management Software (PMS) for attorneys has provided solutions to organizing the date management problem in the office. From the intake information to the final billing, PMS will consolidate every part of the expert's work. The only part of everyday work that is not included is Marketing and Internal Finances, which is necessarily separate from the work that is performed in the forensic lab.

The purpose of this presentation is to stimulate the thought processes of the private practitioner toward streamlining and consolidating his or her work product so it is searchable for future projects and for conflict checks, available for other cases that involve similar issues or questions, and provide an accessible database for historic work that could be useful as resources.

This presentation is an introduction to the software used in law offices as it applies to expert witness work. While not an endorsement of any one brand of software, this presentation will discuss the potential for every expert to consolidate his or her data, evidence, work product, time tracking, and communications into one easy-to-use location that is more secure than the open internet. Each of the software applications that are being presented have been used and there will be discussion on the pros and cons of each. Other software options will be included, but all software in this genre are not necessarily mentioned.

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**Practice Management, Evidence Management, Private Practice Software**



## J17 Forensic Document Examination: Early Incorporation of the Forensic Intelligence Paradigm

Samiah Ibrahim, BSc\*, Ottawa, ON K1S 1R1, CANADA

**Learning Overview:** After attending this presentation, attendees will better understand the relatively new academic pursuit of forensic intelligence, as well as gain insight into how, throughout the history of forensic document examination, laboratories have practically worked with this concept.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community because, ultimately, attendees will take away a sense of how they can apply the principles of forensic intelligence into their research and casework.

Forensic science is simply defined as the application of scientific knowledge to legal matters. Traditionally, the results of forensic analyses are used to help resolve criminal investigations; however, with modern advancements, many of the scientific techniques used in forensic analyses are now common and necessary parts of investigations in both the private and public domain and can be applied from the earliest moments.

Intelligence is the end result of the collection and subsequent analysis of information assembled from myriad sources. The purpose of gathering intelligence relates directly to the directive or mandate of the organization collecting the information. Worldwide, intelligence-gathering agencies draw upon the gamut of their resources in order to render a more complete model during investigations. The use of science and, in particular, forensic science, has long been a part of these resources; however, this does not preclude other organizations from employing forensic analyses in the development of their intelligence product.

Forensic intelligence has been defined in the literature as the accurate, timely, and useful product of logically processing forensic case data for investigation and intelligence purposes.<sup>1</sup> This literature, courtesy of various academic groups working to establish processes and models to exploit forensic case data, highlights excellent examples of intelligence derived from the various forensic disciplines.<sup>2-7</sup>

Forensic examination of the materials, substances, and objects seized from seemingly random or disparate instances or events may identify links between suspects, activities, or items and their involvement in criminal activity. This approach, to look to the collective data generated by these many forensic analyses in new ways to discern non-obvious linkages, will bolster our current practices to create better product and ultimately make better, more-informed decisions throughout the international law enforcement continuum. Ultimately, a mature forensic intelligence approach may provide data prior to an event, particularly in a criminal environment with a serial/recurring component.

This presentation will introduce the topic of forensic intelligence and establish how forensic document examination, from the earliest times, has incorporated these ideas into casework. Further, over the past many years, various data collection tools have been developed by forensic document laboratories around the world with the express purpose of providing ways to link disparate casework. This presentation will highlight some examples of these tools and how they have been used in forensic document examination, as well as to shine a light on the potential to use these same tools to generate data that could input the investigation of other crimes not typically thought of as being in the realm of forensic document examination.

### Reference(s):

1. Olivier Ribaux et al. Intelligence-led crime scene processing. Part I: Forensic intelligence. *2010 Forensic Science International*, vol. 195, no. 1-3: 10-16.
2. Simon Baechler, O. Ribaux, and P. Margot. 2012. 2012 Student Paper: Toward a Novel Forensic Intelligence Model: Systematic Profiling of False Identity Documents. *Forensic Science and Policy Management*, no. 3: 70-84.
3. Quentin Rossy, S. Ioset, D. Dessimoz, and O. Ribaux. 2013. Integrating forensic information in a crime intelligence database. *Forensic Science International*, no. 230: 137-146.
4. Alastair Ross. 2014. Elements of a forensic intelligence model. *Australian Journal of Forensic Sciences*, vol. 47 no. 1: 8-15.
5. Marie Morelato, J. Broseus, A. De Grazia, M. Tahtouh, P. Esseiva, and C. Roux. 2018. Forensic drug intelligence and the rise of cryptomarkets. Part II: Combination of data from the physical and virtual markets. *Forensic Science International*, no. 288: 201-210.
6. Anna Agius, M. Morelato, S. Moret, S. Chadwick, K. Jones, R. Epple, J. Brown and C. Roux. 2018. Using handwriting to infer a writer's country of origin for forensic intelligence purposes. *Forensic Science International*. no. 282: 144-156.
7. Anne Bannwarth, M. Morelato, L. Benaglia, F. Been, P. Esseiva, O. Delemont and C. Roux. 2018. The use of wastewater analysis in forensic intelligence: Drug consumption comparison between Sydney and different European cities. *Forensic Sciences Research* 2471-1411.

### Forensic Document Examination, Forensic Intelligence, Intelligence



### J18 Crossing Borders: Issues From Inter-Jurisdictional Casework

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**Learning Overview:** After attending this presentation, attendees will be aware of the inter-jurisdictional nature of questioned document examination casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating attendees about the challenges and limitations that are associated with inter-jurisdictional work in forensic document examination.

Forensic science has been inter-jurisdictional in nature from earliest times in the various disciplines. As one of the original forensic disciplines, Questioned Documents has myriad examples of this. Some of the earliest reported cases of forgery are accounts of counterfeit travel documents from the far reaches of the world. In approximately 450 BC is the first mention of a written letter from a Persian official requesting safe passage from “the governors beyond the river” as he crossed their lands.<sup>1</sup> Consequently, throughout history, documents such as laissez-passer, passports, currency, and other official documents from foreign jurisdictions have had to be forensically examined to determine authenticity and veracity.

In more modern times, there has been a broadening of the inter-jurisdictional nature of forensic work to include training and other collaboration. Forensic document examination has matured as a discipline over the past century in North America, Europe, and select other areas of the world. Recently, there has been a concerted effort to promote international standards in the examination of documents, including providing training to forensic scientists from all corners of the globe. Further, venues such as the American Academy of Forensic Sciences and other forensic conferences provide opportunity to share knowledge across borders. This learning is multi-path as traditional examiners learn equally from examiners of foreign scripts, for example. It has become commonplace to receive requests for forensic document examination services from foreign entities, about foreign entities, and concerning documents of foreign origin, source, manufacture, etc. This may bring certain elements of challenge and limitation to the examination.

Another issue in working on inter-jurisdictional casework involves the basic logistics of physically receiving the case (continuity of evidence included), or establishing where items will be examined. This may perhaps involve remote field examinations that pose other specific challenges from technical (such as power supply and ventilation) to space requirements and security.

There are examples of inter-jurisdictional cases that involve the prosecution of suspected war criminals and those charged with crimes against humanity in which security concerns add a real element of danger that may be foreign to the traditional forensic document examiner.

A daunting challenge to inter-jurisdictional work is the assurance that the forensic opinions issued in a report are received and understood with the same weight as they were written and intended. Clarity of message is crucial in all forensic reporting; this challenge is increased considerably if there are differences in language, legal system, customs, and culture.

Each of these challenges will be touched upon in this presentation by those who have worked in some regard on the international scene. With the increase in inter-jurisdictional casework, it is important to share experiences within the forensic document examination community so that our work is clearly understood by clients and stakeholders, regardless in which and for which jurisdiction the work is conducted.

**Reference(s):**

<sup>1</sup>. Holy Bible, New International Version, *Nehemiah 2: 7-9*.

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**Forensic Document Examiner, Inter-Jurisdiction Forensic Science, International Forensic Science**



### J19 Developing a Frequency of Occurrence Proportion-Based Database in Forensic Science: A Template Using the Handwriting Database

*Thomas W. Vastrick, BS\*, Apopka, FL 32703*

**Learning Overview:** The goal of this presentation is to provide attendees with a template for how to create a frequency of occurrence database for their location and for their discipline through the experiences and lessons learned developing a handwriting database over the past ten years. Attendees will learn about each step used by one of the founders and leaders of the template project.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by knowledge transfer of the experiences gained through the entire development process of one such database. As such, the worldwide forensic science community will have the benefit of the successes and failures in methodologies utilized and the reasons behind the results.

In 2010, a team of forensic document examiners and statisticians undertook to develop, from scratch, a database of frequency of occurrence proportions. This project was funded by the National Institute of Justice, United States Department of Justice with a research grant. The results were formally presented at the 2015 American Academy of Forensic Sciences Annual Scientific Meeting in Orlando, FL. Subsequent to that, team members headed two further studies that broadened the database to include numerals. To date, there are more than 800 features in the database.

Because there had been few similar studies ever conducted, the team needed to establish several protocols. These protocols, by design, were based on statistical standards as it was determined that best practices dictated that this was to be a statistically driven study concerning forensic science rather than a forensic science study using statistics. The protocols incorporated published information and procedures utilized in other areas. The areas of procedures that were addressed included population sampling, quality control, data selection, system development, reporting, potential for abuse, and integrity of the data.

The statisticians employed a multi-strata system of population sampling based on factors that had been published concerning intrinsic and extrinsic factors that affect handwriting. The team reviewed each factor and determined the best method to address the factors individually. Next, the team used recent census reports to establish the strata goals, set at 80% of the census numbers. The other 20% were permitted to be collected randomly in order to take advantage of randomness and to further address factors that may not have been considered. The population sampling method will be addressed in great detail in this presentation and implications to developing sampling protocols for regions that may have significantly less homogeneity will be discussed.

Another aspect of population sampling was the physical collecting of specimens. Again, standard methodologies needed to be followed and the method with which the team executed this aspect of the study will be discussed in detail.

Data selection was a complex process that required a significant amount of time and processes. First, features were initially selected by subject matter experts based on being deemed objective in nature, as opposed to subjective. The reason was to establish reproducibility in data entry from the many data entry personnel. The method of testing involved use of an Attribute Agreement Analysis (AAA). This presentation will go into the published AAA standards and both the successful and unsuccessful methods used in its implementation.

The system developed for the input and use of the data was complex and unique. Efforts were made to create a system that could be expandable and flexible so that future additions would be easy and use in actual casework could be applied on a national level of targeted subgroups. This presentation will discuss this in detail and demonstrate the various software programs that were developed.

Since population sampling is not precise to the entire population, but rather an estimate, it is important to know how to report results in the most accurate manners possible. This presentation will discuss how the handwriting database deals with this issue.

Finally, procedures were created to minimize the potential for any bias by the separation of duties and the restricted access to data by the forensic specialists. Conversely, the entire team contributed to ideas of potential misuse and abuse of the process and agreed that it was important to address these issues upfront. These issues will also be discussed in the presentation.

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#### Frequency, Statistics, Database



### J20 Statistical Analysis of Handwriting: Probabilistic Outcomes for Closed-Set Writer Identification.

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**Learning Overview:** The goal of this presentation is to provide insights into features of handwritten documents that are important for statistical modeling with the task of writer identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing ongoing forensic statistics research to practitioners in the field. Collaboration and communication is fundamental to the success of emerging research. All statistical methods and code are open-source and available to the community.

The objective of this work is to develop a statistical model that takes all styles of writing (cursive, print, and connected print) and gives a probabilistic evaluation of a closed-set writer identification as well as variability estimates. Such probabilistic statements have potential to support examiner conclusions and be used by the community to provide free and open-source methods for searching a collection of documents.

When forensic examiners compare handwritten evidence, they often focus on small details of writing. Likewise, this statistical approach to the comparison of such documents begins by decomposing writing into small meaningful connected pieces of ink, often corresponding to letters. The R package *handwriter*, developed at the Center for Statistics and Applications in Forensic Evidence, handles this processing task. The small pieces are treated as graphical structures, and graphs are grouped using a novel dynamic method that is based on the similarity of their shapes. The frequency at which graph types appear in writing, along with measurements taken on the small graphs, serve as data for a Bayesian hierarchical model. This study considered measurements such as slopes, lengths, centroid locations, and loops and compared the measurements within groups to investigate their ability to discriminate between writers. Measurements that separate writers well in a numeric capacity will be useful for the statistical model.

There are a few notable insights that arise through the modeling process. First, the model that takes all styles of writing as inputs is able to separate print and cursive writings through a latent variable. Next, through a multivariate over-dispersion calculation, one can evaluate intra-writer variability as it relates to the model.

The statistical model is ultimately used to perform a closed set writer identification analysis. Data for the analysis come from the first 100 writers in the Center for Statistics and Applications in Forensic Evidence (CSAFE) handwriting data collection. In this data collection, study participants were asked to transcribe three writing prompts: the “London Letter,” a short excerpt from *The Wonderful Wizard of Oz*, and an even shorter common phrase. Each prompt was written three times during each of three data collection sessions. This study investigated writer identification performance under varying amounts of known and questioned writing.

*This work was partially funded by the CSAFE through a Cooperative Agreement between the National Institute of Technology (NIST) and Iowa State University, which includes activities carried out at Carnegie Mellon University, University of California Irvine, and University of Virginia.*

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**Statistics, Questioned Documents, CSAFE**

## J21 The Creation of Forged Promissory Notes Using the Signature Which Was for Different Purposes: A Case Report

*Dilara Oner, MS\*, Istanbul University-Cerrahpasa, Istanbul, Fatih 34098, TURKEY; Abdi Ozaslan, MD, Istanbul University-Cerrahpasa, Fatih, Istanbul 34098, TURKEY; Gursel Cetin, PhD, Istanbul University-Cerrahpasa, Istanbul, Fatih 34100, TURKEY*

**Learning Overview:** After attending this presentation, attendees will have seen an interesting example of one of the forgeries in the field of forensic document examination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by creating an example that will raise awareness in the field of forensic document examination.

In recent years, frauds have been frequently encountered by printing promissory notes on the upper side of a signature that was written for a different purpose and unjustly charging the person. In a case given by the court to experts, a forged promissory note was arranged to use the space on the right side of the signature for other purposes. Therefore, the case was found interesting and useful for presentation and discussion.

At first glance, the localization of the signature in the document subject to review is remarkably close to the left edge. In addition, when the signature is compared to the other signatures of the person, it is seen that the signature is left-leaning. In the examination, it was found that the top and bottom edges of the document were 20.5cm and the right and left edges were 9.6cm. When the top and right sides of the document were examined under a stereomicroscope, it was found that the images were compatible with each other and the signs of wear were relatively high. In addition, when the bottom and left sides of the document were examined under a stereomicroscope, it was found that the images were compatible with each other and the signs of wear were relatively low.

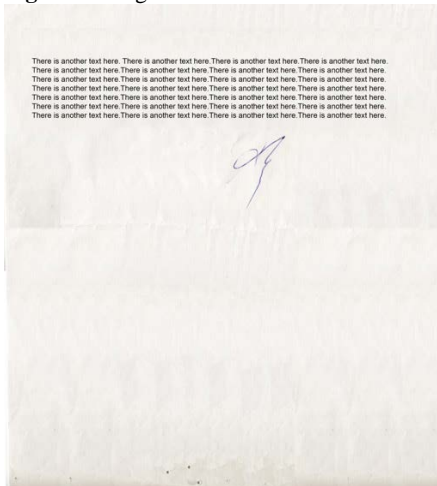
**Findings:** It was seen that there was text on the upper side of a paper whose size cannot be determined at present, and the lower right side of this text is signed as approving the text (Figure-1a). Then the text was turned 90 degrees to the left (Figure-1b) and the paper was cut between the signature and the text. The paper was cut again by the bottom of the signature (Figure-1c), a blank signed paper (Figure-1d) was added to the space at the top of the existing text, and, as a result, the document was made available. (Figure-1e).

In the Turkish legal system, a document must contain certain elements in order to create a promissory note; however, there is no standard regarding the size, color, and printing format of the promissory note. Therefore, in cases where there are elements such as the date of arrangement, the due date, and the amount of money, all types of papers and sizes will be valid. In this way, a forged promissory note can be produced by using a signature on blank paper that was written for other purposes. If there is too much space between the text that is located on top and the signature, between the signature and the bottom of the paper, or on the sides as in this case, the paper can be cut from the top, bottom, or sides, and a forged promissory note can be printed on the rest of the blank.

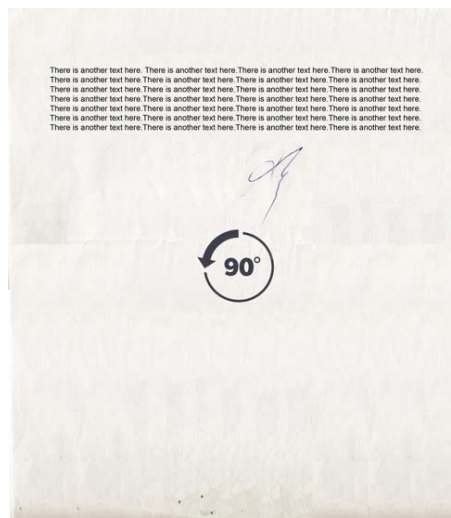
As in the case presented, the person who qualifies as a debtor in such a situation can state that he/she did not sign such a bill and declare an objection to the signature. When the expert's examination, commissioned by the court, naturally states that the signature belongs to him/her, a complex situation arises. Therefore, the court and the experts in counterfeiting should be careful and focus on not only the objection to the signature.

In particular, all documents that are not printed or printed with computer printer output, electric typewriter, typewriter, or handwritten should also be examined in terms of the cut edges of the printed paper, the layout format of the promissory note, text, line ranges, and whether toner waste in laser printer output is above wet-signature lines.

**Figure-1:** Stages of creation of a counterfeit document



**Figure-1a:** The text above is signed by the person



**Figure-1b:** The paper is turned 90 degrees to the left







### J22 Writing Instrument Developments: Hybrid Pens, Rollerball Pens, and Mixable Fountain Pen Ink

*Tobin A. Tanaka, BS\*, Canada Border Services Agency, Ottawa, ON K2E 7M6, CANADA*

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**Learning Overview:** After attending this presentation, attendees will be aware of three evolutions in writing instruments: (1) hybrid pens; (2) roller ball pens that are compatible with fountain pen ink; and (3) mixable ink for fountain pens. Examples of the physical and optical characteristics of these pens and inks when examined with a variety of instrumentation including hand magnifiers, microscopes, spectral examinations, and scanned images of the writings will be shown.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of these developments in the writing instrument industry. This presentation will provide information on the features that may be observed if examining such writings or inks.

Far from being a static industry, the writing instrument world continues to develop new and evolving products that may be used in writing. Although document examiners may not always need to precisely identify the type of writing instrument used in the production of writing, there is a need for an awareness of the ever-increasing types of writing instruments in use. Standards, such as those from the Scientific Working Group for Forensic Document Examination (SWGDOC) and the European Document Experts Working Group (EDEWG) outline some instances in which observations may disclose evidence that must be considered as part of an examination.<sup>1-4</sup>

Hybrid pens utilize a combination of ballpoint and gel ink. The intention of the manufacturer is to provide a longer-lasting ink supply typical of ballpoint pens, with the benefits of smooth writing as often experienced with gel pens. Differentiating between hybrid pen lines and those of ballpoint or gel can be very challenging.

Most rollerball pens are supplied with rollerball refills, but a few are designed to take fountain pen ink. Even when chemical analysis is permitted, there is the potential for misinterpretation of the writing instrument type if too much reliance is weighted on the chemical composition alone.

Ink manufacturers advise against the blending of fountain pen inks. The reason being that blending different inks may result in coagulation of the ink and clogging of the pen; other more adverse chemical reactions have been known to occur. Mixable ink is formulated to permit the blending with variable proportions to make a wide variety of desired colors.

This presentation will provide some indication of what features may be reliable indicators of hybrid ink writing and rollerball pens that use fountain pen ink. For mixable fountain pen inks, this presentation will highlight how well the inks blend together, the homogeneity of any such mixture, and the evidence that may indicate to a document examiner that the written line may be the result of blended fountain pen ink.

**Reference(s):**

1. SWGDOC. *Standard for Test Methods for Forensic Writing Ink Comparison.*
2. SWGDOC. *Standard for Writing Ink Identification.*
3. SWGDOC. *Standard for Examination of Handwritten Items.*
4. European Document Experts Working Group (EDEWG). *Examination of Alterations.*

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**Writing Instruments, Fountain Pen Ink, Hybrid and Rollerball Pens**



### J23 Detecting Backdated Documents Through Line Layout Approaches to Font Identification

*Thomas W. Phinney, MS, MBA\*, Portland, OR 97206*

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**Learning Overview:** After attending this presentation, attendees will learn the utility of font identification in detecting backdated documents. Attendees will learn about font dating and its use in detecting backdated documents, as well as both traditional methods of font identification and newer methods based on glyph spacing, which are suited to automation and capable of yielding results even with very poor document reproduction.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by not only covering widely used tools but also giving attendees new tools for detecting document backdating and identifying an area for more research.

**How Font Identification Can Demonstrate Forgery:** Identifying a font used in a document, when combined with information about when the font was designed and made available to the public, can be supportive or conclusive in proving that the document was not created on a date claimed. This can not only prove backdating, but in cases where the purported author was deceased before the font was available, can be evidence of forgery.

**Traditional Methods and Resources for Font Identification:** A variety of books, manuals, and web sites provide visual references and information on font classification and earmarks. Many of these approaches require some degree of expertise for reliable results.

**Complications for Font Identification and Timelines:** Although a font may be officially released on a given date, that does not mean it was completely unavailable before that date. Especially for fonts bundled with operating systems and office suites, the availability of pre-release and beta versions can complicate the story by creating a gray area of time, when a new font was not yet used by the average person, but still available to some people outside the company. Calibri illustrates this issue, with its increasing availability to outside testers for a period of almost two years before its formal and full release with Windows® Vista and Office 2007.

Another complication, for at least some common typefaces, is the existence of (later) lookalike fonts, sometimes called “clones” or “knockoffs” of the original. This generates the potential for false negatives, or less commonly, false positives.

**Line Layout Methods for Font Identification:** Except for traditional typewriters and fonts of that same style (such as Courier, Prestige Elite, and Consolas), wherein all letters take the same amount of space, most fonts are “proportional”: each letter is allocated a different amount of space. Aside from some proportionally spacing typewriters and a few fonts created to imitate the spacing of earlier fonts, the spacing of fonts is generally unique, like a fingerprint.

This spacing is revealed by cumulative character width, especially with non-justified (ragged right) text. Even with a very poor document reproduction, one can use this method by comparing line breaks and especially ragged line endings. Any large-enough sample of such lines is like a fingerprint: the relationship of where each ragged line ends, relative to the adjacent lines, uniquely identifies a particular proportional font. Because this method is based on *relative* line endings, it does not require duplicating the original document and is unaffected by distortions that can occur when documents go through copying, faxing, scanning, etc.—even multiple generations of reproduction.

An employee of Adobe® at the time made this observation in 2004, in regard to the Bush National Guard memos. This concept, when applied in an automated way by a computer program, became the basis of a patent application. The concept was subject to internal peer review, and the patent was granted by the United States Patent & Trademark office and expires in September 9, 2025).

That this technique can differentiate different fonts has been thoroughly demonstrated on a small scale, with handfuls of fonts at a time. However, no testing has been done on a larger scale, to further validate the methodology or determine the statistical reliability of the method. Such testing can be done manually; if automated, it would presumably require either licensing the patent or waiting for patent expiry in 2025.

*The presenter has no financial interest in Adobe® Systems nor in licensing the patent.*

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### Forgery Detection, Font Identification, Document Authentication



### J24 The Interaction of Writing Profiles and Automated Scoring Rules

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**Learning Overview:** After attending this presentation, attendees will better understand the importance of statistical invariance as it relates to the development of automated scoring rules for handwriting verification systems. The Random Non-Match Probability (RNMP) is the chance that two writing samples written by the same writer are declared a non-match by a given biometric scoring method. Some important questions that arise are whether or not this chance is the same for every writer in a population of writers and whether or not it is affected by the number of words in the writing samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by delving into the design and validation of automated handwriting identification systems to writing samples, the statistical methods, and interpretations based on scores.

**Hypothesis Statement:** Designed studies of handwriting will allow researchers to identify scoring rules that have an RNMP that varies as a function of a writing profile.

In the construction of Automated Handwriting Identification Systems (AHIS), pairwise scoring rules play an important role associated with characterizing the performance metrics of the system. Most AHIS for forensic source identification are optimized to recommend the order in which an examiner should search a candidate list of known writers to identify the source of a writing sample with an unknown writer. In effect, they recommend the source of a writing sample that an examiner will then verify. A natural measure of the performance of a scoring rule is the associated Random Match Probability (RMP). The RMP is the chance that two writing samples from different writers are declared a match—this is analogous to the “likelihood of a chance match” in the logical approach to handwriting analysis. Unfortunately, most handwriting AHIS are not optimized for minimizing the RMP and are instead focused on minimizing the time to find a writer in database of writers. This means that, if one wishes to compare two writing samples in a head-to-head comparison to measure the similarity with a given AHIS, then the researchers will usually need to design a strategy for encoding the output of the system in order to develop an interpretable scoring rule (by interpretable, this study means that a score has meaning that is consistent across writing profiles, or that the RNMP for two writing samples written by writer A is the same as two writing samples written by writer B). This work will discuss various strategies for constructing pairwise dissimilarity scores and characterizing the performance of the resulting scoring rules.

Using writing samples collected under a National Institute of Justice (NIJ) -funded research project, this study explores the properties of various pairwise scoring rules for the results of the FLASH ID system. This study also focuses on the scores’ dependence on the amount of handwriting (in each writing sample) that is made available to the system. Another important focus of this research is on the visual display of the statistical methods to convey the performance of the scores.

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#### Source Identification, Handwriting, Biometric Performance Metrics



### J25 Writer Classification of Handwritten Characters Using a Neural Network

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**Learning Overview:** After attending this presentation, attendees will understand the results of writer classification using a neural network and the necessity of the future research on a neural network using various data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the validity and the problems of the application of a neural network to handwriting identification.

Machine image recognition technique along with deep learning has been advancing rapidly, and its application to forensic document examination has also been expected. A writer classification experiment using a neural network, which was conducted as one of the feasible studies, is reported in this presentation.

Writer classification using the neural network LeNet was conducted. Samples used for the experiment were 26 uppercase Latin alphabetical letters, 26 lowercase letters, 26 Hiragana characters, and 26 Kanji characters, written five times respectively by ten people. Each letter and character was originally written in a 1cm x 1cm square box in handprint. Then, an 8-bit grayscale image of each sample was obtained under conditions in which the image size was 32 pixels x 32 pixels with a resolution of 85 dots per inch (dpi).

Writer classification using LeNet was conducted on the following four categories: uppercase Latin alphabetical letters, lowercase letters, Hiragana characters, and Kanji characters. Image data were divided into two groups: training data and validation data. Four out of five samples per letter or character were used for training and the remaining one was used for validation. Thus, there were 1,040 samples (26 characters x 4 samples per character x 10 writers) used for training and 260 samples (26 characters x 1 sample per character x 10 writers) used for validation per category. Training was done under conditions in which the batch size was 64 and epoch was 50. After training was completed, validation samples were classified into any of ten writers respectively, and the accuracy ratio was calculated on four categories.

The accuracy ratio was 0.7615 for uppercase letters, 0.6500 for lowercase letters, 0.7500 for Hiragana characters, and 0.7769 for Kanji characters, which was higher than expected. The average accuracy ratio of respective letter or character (training samples: 4, validation samples: 1, batch size: 8, epoch: 50, trials: 104 (26 letters or characters x 4 categories)) was 0.5897, and the expected accuracy ratio of each of the four categories was around 0.7000 before the experiment. These results may show the importance of the number of samples in the application of the neural network. Recall ratio per writer varied the largest in lowercase letters (0.0769–0.9230), which explained the reason of the lower accuracy ratio. In these classification experiments, feature extraction of individuality from each writer's handwriting was conducted on all the training samples, and this means a writer's features extracted were expected to be common to all letters or characters used. Thinking that the comparison of documents in forensic document examination is performed on the same letters or words and that forensic document examiners have knowledge and experience with the feature extraction from one's handwriting, these results are interesting. The results may suggest that a person writes various letters and characters in a constant and unique manner.

Two writers showed a high recall ratio (over 0.9000) in all four categories, and their handwriting samples were unique in size, shading, or the location of the handwriting in the box, which may be the primary features for the neural network classification in this experiment. Forensic document examiners do give weight to these features, but they give more weight to graphic features of a handwriting, such as slope, angularity, relative relationship between strokes in writer classification, or identification tasks.

Several findings on the application of a neural network to writer classification were obtained from these experiments. More experiments, such as classification using samples of the same graphic form with augmented samples, using image data with higher resolution, or using more complicated algorithm, will be necessary for the future study.

#### Handwriting, Writer Classification, Neural Network

## J26 Availability of Measurement of Ascender and Descender Parts of Letters in Determining the Gender of the Writer

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**Learning Overview:** After attending this presentation, attendees will have gained information about the results obtained in a study in Turkey about the measurement of certain parts of letters, which is one of the methods used in determining whether a manuscript belongs to a woman or a man, and how the possibility of applying the same method will arise in different countries.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about the tests of a study conducted in Turkey based on numerical values and usability to determine whether a manuscript belongs to a woman or a man.

One of the findings used when determining whether a manuscript belongs to a female or a male is to compare the length ratios of the parts by measuring some letters. The goal of this study was to determine the differences between female and male writings by measuring the parts of the same letters in the same words on the same texts written by females and males.

For this purpose, manuscripts that were written by 20 females and 20 males whose age range was 22–63 years (average 32.7 years) and whose educational background was either university students or graduates, who were Turkish citizens living in the same geographic region (Istanbul, Turkey) were analyzed in Adobe® Photoshop® software using the letter “d” in “başladı, derken, uyandım” words, the letter “t” in “lokantada, seyrettik, papatyalarım” words, the letter “p” in “akıp, papatyalarım” words, the letter “k” in “yaklaşan, sığındık, katıldı” words, the letter “y” in “dinmesiyle, yol, ayrıldım” words, the letter “b” in “İstanbul, boğaz, bulutlar” words, the letter “f” in “ferah, film” words, the letter “g” in “gibi, gökyüzü, gitmeye” words, and the letter “h” in “sabahına, hoş, bahar” words. The data obtained were analyzed with *t*-test in SPSS 23 software.

It was found that ascender and descender parts of all letters were the higher length value in male participants and the difference was statistically significant in all letters except “y,” “f,” and “g” (p value <0.05).

It is stated in related literature that the handwritings of females and male differs from each other.<sup>1,2</sup> Objective findings to be used to reveal this difference should be based on numerical measurements. In this study, significant differences were obtained in some selected letters. Also, if the measured number of letters, the range between words, the range between letters, and the number of measurements is further increased, much more reliable findings can be obtained in the differences between females and males.

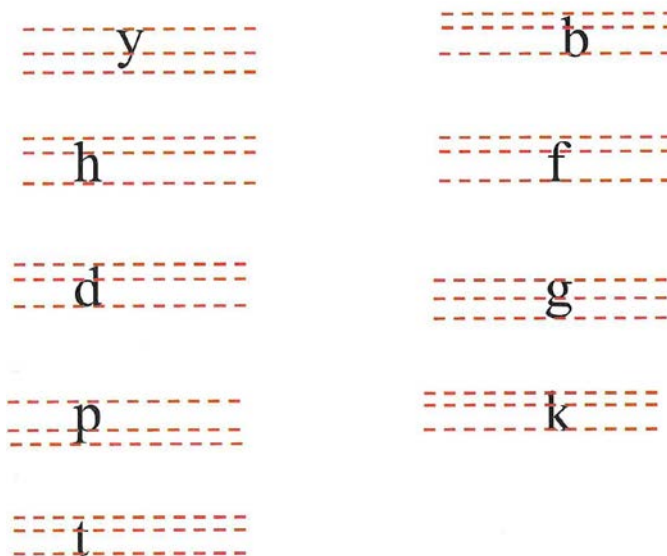


Figure 1: Measurement zones in letters

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### Female Handwriting, Male Handwriting, Gender Determination



### J27 A Study of the Impact of a Primary Learned Handwriting Language on a Secondary Language

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**Learning Overview:** After attending this presentation, attendees will be able to understand how a primarily learned handwriting language affects the handwriting in the secondary learned language. The significance of the analysis is further represented with the help of various statistical tools.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a key aspect in the field of handwriting examination. This is the prevalent aspect in the field because people around the globe are being attracted to learning new languages, but their native language always has some impact on the later learned language.

Identification of handwriting found on a disputed document by comparison with the known handwriting samples of the suspect still comprise the problem that is most commonly referred to a forensic document examiner. One of the important scientifically established principles that govern such analysis and identification is the “Principle of Comparison,” which explicitly states that, for obtaining correct results, like has to be compared with like; meaning, thereby, that the expert has to analyze and rely upon similar letters and combinations between the questioned and the standard handwriting samples and, consequently, the problems in which similar handwriting samples in the same script have not been provided for comparison usually fall outside the scope of forensic document examination. However, in this field, like any other human activity, perfect and ideal conditions are hard to achieve.

Handwriting is an acquired motor skill and combined output comprised of visual perception and activity, comprehension of form, activity of the central nervous system, the anatomy and physiology of bones, muscles of the hand and arm, and, most commonly, the script used by a person in common and its effect on his secondary language. Linguistic diversity among different world populations is a well-established and recognized fact. Throughout the world, thousands of languages exist that are written in hundreds of different scripts.

As we know, India is a polyglot country as there are a total 122 languages and 234 mother tongues. Only 22 languages have gained official status by the Constitution of India. From prior studies, it was demonstrated that the mother tongue has a great impact on the language that we learn later in life. In a similar way, the language that one uses primarily for writing purposes affects the secondary language. Rendering an expert opinion has become highly difficult and problematic in cases in which a questioned document in one language and a standard document in another language are available for comparison. Handwriting examination in these types of cases is of foremost importance because these features may lead to the nationality and locality of the writer of the handwriting in question and may help the document examiner compare the handwriting samples, irrespective of the script.

This study is basically focused on the similarities found in the class as well as individual characteristics of the Mother tongue and secondary learned language. One hundred individuals were selected for the study and eight handwriting samples for both languages (Punjabi [the Mother tongue of the Punjab region] and English) were collected with the consent of the subjects. Results obtained were statistically analyzed, and it was revealed that an earlier learned language affected the secondary language significantly. In 68% of the cases, there is a similarity between the individual and class characteristics of the two languages. Apart from this, the given study also reveals that script independent comparison of handwriting is possible when basic elements of strokes (for instance, grapheme) are considered.

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#### Language, Script, Primary Language



### J28 Comparing Latin With Cyrillic Script in Handwriting Identification

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**Learning Overview:** After attending this presentation, attendees will better understand handwriting analysis of documents written in different alphabets.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a unique study in which documents written in different alphabets were analyzed as well as by teaching attendees about the methods for handwriting and signature examination of documents written in different alphabets.

Identifying handwriting has always represented a challenge as there is no such instrument that could identify the writer in a clear and unambiguous way. In fact, identification has to be made by a handwriting expert, while instruments are used only as tools in his or her work. Even though forensic examination of handwriting has an established and worldwide accepted methodology; its application may differ depending on the script that is examined. The goal of this presentation is to examine the possibilities of handwriting analysis of documents written in different alphabets (Latin vs. the Cyrillic alphabet, and vice versa).

The professional standards used for evaluating handwriting and signatures in the Latin alphabet can also be applied to those in the Cyrillic alphabet. The most important element is the movement of a writing instrument on a writing surface, leaving a trace on the paper that has an important role in identifying the writer.

When an expert witness analyzes text in the Cyrillic alphabet, such as Russian, Serbian, Bulgarian, Ukrainian, or other languages in which some letters are similar to the letters of the Latin alphabet, while others are completely different, he or she has to take into consideration all possible variations of the alphabet. Also, the fact that in the Cyrillic alphabet, it is more difficult to evaluate printed than cursive letters, so the expert has to have access to writing samples from that particular country or region.

The Croatian Latin alphabet has 30 letters, and 27 of them contain one character only. The Cyrillic alphabet has 30 to 40 letters, depending on the language using it. If the lists of letters of both alphabets are compared, one can notice that there are also letters whose form is the same, both in the Latin and Cyrillic alphabet, but they are pronounced in different ways. However, the fact that there are some letters that are the same but have a different meaning makes the comparison of two alphabets easier.

In order to answer research questions, testing and an analysis have been conducted of the handwriting of 12 volunteers from the Vukovar area (a locale in Croatia near the Serbian border) who use both alphabets on a daily basis. In order to collect handwriting samples, every person was asked to copy the same text both in the Latin and the Cyrillic alphabet. Every sample in the group written in the Latin alphabet matched the sample in the Cyrillic alphabet that had been written on the same day with the same instrument, consisting of exactly the same text.

In the first phase of the research, a preliminary analysis was performed to check whether the collected handwriting samples meet handwriting analysis professional standards regarding their quality and quantity as is the standard practice under International Organization for Standardization (ISO) 17025. Next, a detailed examination of general and individual handwriting features was conducted on all handwriting samples written in the Latin and the Cyrillic alphabet for each person individually.

The above-mentioned features of the Latin and the Cyrillic alphabet handwriting samples were examined in terms of their degree of similarity as well as dissimilarity using the comparative method. A stereomicroscope and video spectral comparator were used to compare handwriting samples as well as for the capture of images and for measurements of the size of letters and handwriting in general. Each feature's degree of similarity and dissimilarity was rated by two experts independently. The obtained results have shown that it is possible to evaluate samples of handwritten text written in different alphabets. However, when interpreting the results, one should take into consideration all the limitations suggested by handwriting and signature analysis professional standards, (e.g., photocopies, quality and quantity of materials, similarities in handwritings of various writers, diversity of the content, etc.).

Finally, it should be emphasized that the present research is only a case study. In order to draw universal conclusions, one would have to conduct an experiment on a large group of individuals and real case subjects.

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#### **Handwriting Examination, Latin Script, Cyrillic Script**



### J29 The Forensic Document Examiner (FDE) Forum

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the state of forensic document examination through hearing attendee opinions on various current topics. The purpose of this forum is to engage attendees on their thoughts on current issues facing forensic document examiners. Topics will be chosen by the forum presenters and presented to the attendees to then discuss. These topics can range from standard methods to training to opinion terminology. Progress of any discipline depends on feedback from stakeholders.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing members and non-members alike, as stakeholders, the opportunity to provide their thoughts on topics current in the field of forensic document examination.

The concept of the FDE Forum is not new to the Questioned Document community. It was a concept first utilized at the Southeastern Association of Forensic Document Examiners (SAFDE) meetings in the early 1990s as a method of opening up certain topics of concern to their members. At these meetings, there was a discussion starter on subjects upon which there may have been disparate opinions. Through forums of this type in other organizations, there have been many lively discussions on topics such as the strength of opinions on examinations involving photocopies or rubber stamp impressions. Other topics that could be considered are opinion terminology, board certification, standard training, or specific requirements found in our standard methods. A popular theme in the past has been what type of evidence is required in order to achieve a particular conclusion in handwriting examination (for instance, the elimination of a subject).

Not all discussions must necessarily be centered on issues facing forensic document examiners. Some discussions could focus on attendees' thoughts on and interpretations of quotes from texts in the field by authors such as Harrison, Osborn, and Hilton. Other discussions could begin with the opinions of some of these authors, and other discussions could be in criminal and civil cases of notoriety, past and present. This type of forum is an excellent way to poll attendees on their preferences on methods of testimony, court presentations, and laboratory requirements regarding testimony. Any topics that could relate to international issues and our theme "Crossing Borders" would be appreciated.

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#### Forensic Document Examination, Forum, Stakeholders



## K1 Identification of Fentanyl and Fentanyl Analogs by Using High-Resolution Mass Spectrometry and Machine Learning

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**Learning Overview:** After attending this presentation, attendees will be familiar with the fragmentation pattern of fentanyl as well as its derivatives under Collision-Induced Dissociation (CID). Attendees will also be introduced to a new methodology where the structure of major fragmentation ion could be predicted by Konstanz Information Miner (KNIME) workflow.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a novel KNIME workflow that is capable of predicting the major fragmentation ion as well its structural similarity against the reference based on the input tandem mass spectrum.

Fentanyl is a type of synthetic opioid that was originally developed as a pain management agent for cancer patients. Because of its stronger addictive reaction over morphine or heroin, it was diverted into a type of abused drug, which largely contributed to the current ongoing opioid crisis. One special emerging issue regarding the detection of fentanyl is the rapid evolution of fentanyl derivatives, as the analytical standards in the forensic laboratory may not be able to keep up with the development of newer fentanyl derivatives.

KNIME is a free and open-source data analytics and mining platform developed by a team of software engineers at the University of Konstanz in 2004. Compared with other data mining software such as Statistical Package for the Social Sciences (SPSS) and Statistical Analysis System (SAS), KNIME was chosen in the study for its user-friendliness as well as its versatility. Traditional cheminformatics platforms rely on command line interfaces to operate, which considerably hinders their application as they require the operator to have certain training in command-line language. KNIME, on the other hand, has a Graphic User Interface (GUI) and modular functionality, which greatly facilitates the operation as well as the modification of calculation as the operator could change the functionality of the whole workflow by connecting and disconnecting nodes. Datasets obtained from different sources could be integrated into one workflow. Currently, KNIME is widely used in biological as well as pharmaceutical applications.<sup>1</sup>

Regarding the techniques used in this study, quadrupole Time-Of-Flight (qTOF) Mass Spectrometry (MS) was used to obtain Tandem Mass Spectrometry (MS/MS) spectra and the design of workflow was performed on the KNIME platform. MS has become one of the leading techniques in multiple fields, including forensic science, because of its sensitivity and versatility. Reinforced by the capability of performing structure elucidation via CID, hybrid mass spectrometer such as qTOF/MS has become a powerful tool in the study of controlled substances, including fentanyl derivatives. In CID, pure nitrogen gas is pumped into the collision cell where it collides with the targeted ion, causing fragmentation of this ion, which could provide useful information for structure elucidation as different molecules will fragment differently depending on their structure.

Thus, in this study, a novel KNIME-based workflow was created based on a series of CID MS/MS spectra from unmodified fentanyl as well as five additional fentanyl derivatives, including benzyl carfentanil, cyclopropyl fentanyl, cyclopentyl fentanyl, furanyl fentanyl, and acryl fentanyl.<sup>2,3</sup> Preliminary results suggested that two common fragmentation patterns could be observed on the fentanyl ion, including product N-phenylpropionamide ( $m/z$  188) and a neutral loss of cyclohexylamine (149 Da). Across a total of five different fentanyl derivatives, either one of these patterns could be observed and the appearance of fragmentation pattern is related to the structure of fentanyl derivative. Fentanyl could be considered as the structural combination of an N-phenylpropionamide with a cyclohexylamine and functionality modifications could be on either part. If the main product ion of  $m/z$  188 showed up, this fentanyl derivative should have modification on the side of N-phenylpropionamide. If the neutral loss is 149 Da, it should be a fentanyl derivative that had a modification on the cyclohexylamine. By utilizing this observation, a KNIME workflow could be established based on the fragmentation pattern of fentanyl derivatives obtained from MS/MS on an Agilent® G6520B qTOF mass spectrometer. This KNIME workflow was able to read MS/MS spectrum file (in form of a CSV file) from different software vendors and determine the structure of major fragment ion by  $m/z$  and mass difference based on a set of preset rules. It could also predict structural similarity as a numeric factor of Tanimoto coefficient by calculating the proportion of common functional groups between unmodified fentanyl and fentanyl derivative.

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### Fentanyl, Tandem Mass Spectrometry, KNIME

## K2 An Evaluation of Screening for Drug Use Using Postmortem Prolactin (PRL) Levels in Serum and Cerebrospinal Fluid (CSF)

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**Learning Overview:** The goal of this presentation is to show the significance and practicability of measuring PRL as a drug screen by means of a biochemical examination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the utility of drug screening examinations using PRL as an index.

**Introduction:** PRL is a hormone primarily secreted by lactotrophs in the anterior pituitary gland. PRL secretion can be controlled by PRL-inhibiting factors such as psychiatric drugs, including anti-dopamine agents. The use of Dopamine (DA) antagonists to increase PRL levels may lead to hyperprolactinemia under clinical circumstances. On the other hand, as a screening method for drug use, detection of the drug in question has been the main approach to date, and reports using hormone levels in body fluids as indices have been lacking. It is speculated that PRL would offer a marker in autopsy cases with unknown details of drug abuse. The goal of this study was to investigate postmortem PRL levels in serum and CSF as potential markers of drug abuse in autopsy cases.

**Materials and Methods:** One hundred twenty-one autopsy cases were examined, after excluding cases involving acute hypoxia/ischemia such as asphyxia, because PRL concentrations are reportedly increased under hypoxic conditions. Detected drugs were classified as DA antagonists ( $n=10$ ), stimulants (e.g., methamphetamine and amphetamine;  $n=10$ ), psychotropic drugs other than DA antagonists ( $n=23$ ), other non-psychotropic drugs ( $n=28$ ), and no detected drugs ( $n=50$ ). Samples comprised of blood collected from the right heart chamber and CSF. PRL was measured by chemiluminescent enzyme immunoassay. *PRL* gene expression in the anterior pituitary of autopsy cases was analyzed by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). The PRL-positive cell ratio in the anterior pituitary gland was measured by immunohistochemical analysis. Gynecomastia was also evaluated using Computed Tomography (CT), and compared with serum and CSF levels of PRL.

**Results:** Serum PRL levels in the present cases, except for DA antagonist cases, were similar to clinical reference levels (3.1–29.3ng/mL). PRL levels in serum and CSF were higher in DA antagonist cases (serum: range 18.8–200ng/mL, median 41.0ng/mL; CSF: range 11.7–394ng/mL, median 34.1ng/mL) than in other cases (serum: range 1.99–217ng/mL, median 20.3ng/mL; CSF: range 1.23–97.2ng/mL, median 9.26ng/mL). Significant differences in *PRL* gene expression in the anterior pituitary were evident between DA antagonist cases and other drug cases ( $p < 0.0001$ ). However, no significant difference in immunohistochemical PRL-positivity ratio in the anterior pituitary gland was evident between drug-detected and drug-undetected cases. PRL levels in serum and CSF correlated with *PRL* messenger RNA (mRNA) expression in cases with abuse of DA antagonists. No relationships were identified between serum or CSF PRL level and gynecomastia using CT in any groups using drugs.

**Discussion:** Use of DA antagonists increased PRL levels in both serum and CSF from autopsy cases. High RT-PCR expression of *PRL* with high serum and CSF levels of PRL were suggested to be controlled by *PRL* gene expression response. These results suggest that postmortem measurements of PRL may prove useful for diagnosing cases of DA antagonist use.

### Prolactin, Dopamine Antagonist, Drug Screening



## K3 Metabolism and Estimation of Intake of Intravenous Nicotine Injection

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**Learning Overview:** The goal of this presentation is for attendees to better understand the distribution of nicotine in the body and the pathophysiology of acute nicotine poisoning.

**Impact on the Forensic Science Community:** Estimating the details of nicotine intake based on its distribution of nicotine and its metabolite, cotinine, in the body could be expected to be helpful for not only forensics, but also treatment for acute nicotine poisoning.

A rare case of death involving nicotine poisoning through an intravenous injection was experienced; the metabolism of nicotine in the body was analyzed and the intake was estimated based on the results.

**Case History:** A young Asian female was found by her husband in cardiorespiratory arrest in a hotel room. She was brought to the hospital, but was subsequently declared dead. About 36 hours later, a forensic autopsy was performed to identify the cause of death. A syringe, liquid nicotine, and hydrogen peroxide water were found in her hotel room.

**Autopsy Findings:** The subject was an adult female (height: 153cm; weight: 45.3kg). She had dark reddish-purple hypostasis and her face was moderately congested. The palpebral conjunctivae were congested, with few petechiae. Injection marks running along the course of the blood vessel with subcutaneous hemorrhage were seen on the left elbow fossa, left forearm, and right upper arm. Both left and right heart blood contained dark red blood with liquidity. The brain (1,285g) was edematous and swollen, and the lungs (left: 380g; right: 385g) were moderately congested with parenchymal hemorrhage. The other organs were also congested.

**Results and Discussion:** The present case was considered acute poisoning. Toxicologic examination clarified the presence of nicotine, which reached the lethal level in blood. Further examination was performed in other body fluids and organs, and a high level of nicotine was detected in the tissue around the injection mark on the right upper arm. Cotinine, the metabolite of nicotine, was quantified, but a low level was indicated, except for in the tissue around the injection mark on the right upper arm. Hydrogen peroxide was also quantified, and the results were within the normal range of a healthy person. For these reasons, it was concluded that the cause of death was acute nicotine poisoning by intravenous injection. Nicotine absorbed into the body migrates to the liver to be metabolized and discharged from the kidneys. Based on the half-life of nicotine and cotinine, it appears possible to estimate the amount and the time passed since intake, but this is extremely difficult because of individual genetic and racial differences in the metabolism of nicotine. Therefore, much more data must be collected from individual samples and analyzed in terms of the efficiency of nicotine metabolism. The quantification of nicotine and cotinine in cases involving nicotine poisoning could be useful to estimate the amount, route, and time of intake.

### Nicotine, Injection, Toxicology



## K4 A Validated Method for the Quantitative Determination of Anabolic Steroids in Urine by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Overview:** After attending this presentation, attendees will better understand a validated method for the quantification of anabolic steroids in urine by basic hydrolysis, liquid-liquid extraction, and LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing a method validation to rapidly and simultaneously confirm 12 anabolic steroids in urine.

Anabolic steroids are the synthetic analogues of testosterone. These drugs have high abuse potential and are most often used by males to increase muscle mass and enhance athletic performance. Abuse of anabolic steroids can lead to early heart attacks, strokes, liver tumors, kidney failure, and psychiatric problems. Also, stopping use of these drugs can cause depression, which in turn can lead to resumption of use. Unlike many other drugs of abuse, these drugs are often injected; therefore, users who share needles or use non-sterile injecting techniques are also at high risk for contracting dangerous infections such as viral hepatitis and HIV.

Most of the methods for the detection of steroids are currently based on Gas Chromatography/Mass Spectrometry (GC/MS) analysis. These methods involve lengthy extraction procedures followed by derivatization of steroids and metabolites. Additionally, GC/MS methods are used to detect steroid metabolites instead of the parent drug due to their lower sensitivity. An LC/MS/MS method was recently developed for the detection, identification and quantification of 12 anabolic steroids (testosterone, boldenone, fluoxymesterone, clostebol, oxandrolone, formestane, clenbuterol, dihydroepiandrosterone, epitestosterone, methandrostenolone, stanozolol, and 3-hydroxystanozolol) in urine samples. Briefly, the method involved hydrolysis of glucuronide metabolites of steroids using  $\beta$ -glucuronidase followed by liquid-liquid extraction. The dried extracts after reconstitution with organic/aqueous solvents mixture were injected onto an Agilent® 6460 QQQ LC/MS/MS in positive ionization mode. Separation was achieved on an Agilent® ZORBAX® Eclipse® XDB-C18 column (4.6 x 100mm, 1.8um) with a flow rate of 0.3mL/min of 0.1% formic acid in H<sub>2</sub>O (A) and 0.1% formic acid in methanol (B). The gradient was initiated at 50% B, increased to 60% B over 2min, held at 60% B until 9min, increased to 100% B until 12min and held at 100 % B until 15min, decreased to 50% B at 15.1min, for a total run time of 19min. Method validation was conducted according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines.

Sample preparation involves basic hydrolysis followed by liquid-liquid extraction. Good linearity and reproducibility were obtained for all steroids and metabolites with a coefficient of determination  $R^2 > 0.99$ . Limits Of Quantification (LOQ) ranged from 1ng/mL to 5ng/mL for various steroids and metabolites. Excellent imprecision and accuracy were achieved (CV <15%) for all steroids and metabolites. Twenty-five urine samples collected from international competitors were tested using this method. Stanozolol, boldenone, testosterone, epitestosterone, and clenbuterol were the major positive steroids found in this study.

### Anabolic Steroids, LC/MS/MS, Drug



## K5 Cocaine Overdose: A Fatal Record

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**Learning Overview:** After attending this presentation, attendees will better understand how high human tolerance can be to cocaine in chronic consumers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community due to the extraordinary case reported: a blood cocaine concentration of 333mg/l was found, the highest recorded concentration of cocaine ever found in overdose death.

The decedent was a 48-year-old White male who police reported had a long history of cocaine abuse; he was found dead in his bathroom between the toilet bowl and the shower, with the door of his house closed from the inside and no evidence of a break-in. Furthermore, his ex-wife reported that he hid drugs under his shoe insoles. Police also discovered some white powder on a bathroom cabinet, which was determined to be cocaine.

The autopsy revealed pulmonary edema and generalized visceral congestion, no foreign body in the airways, and no violent injuries on the corpse. White crystals were found on the decedent's nostrils. A nasal swab and a pulmonary artery blood sample of 25cc were taken and stored at -20°C. Both samples were submitted to the toxicology laboratory of Bari for analysis; samples of vitreous humor, urine, hair, and abdominal fat were taken for further testing.

Due to appropriate fluidity and the suitable quantity of blood taken, the blood sample did not require any dilution in order to be tested. Standard screening protocols were applied during blood testing: Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID) was used to search for alcohol and the immunochemical method was used to search for drugs (cocaine, methadone, cannabinoids, opiates, barbiturates, benzodiazepines, amphetamines, and tricyclic antidepressants). Positive results were found for cocaine and further testing was conducted by Gas Chromatography/ Mass Spectrometry (GC/MS); a blood concentration for cocaine of 333mg/l, Ecgonine Methyl Ester (EME) of 35.1mg/l, and Benzoyllecgonine (BE) of 69.1mg/l was reported. Testing on the nasal swab by GC/MS/FID resulted positive for cocaine, EME and BE, confirming that the drug was administered (or possibly self-administered) intranasally.

The outstanding concentration observed prompted a review of scientific literature to compare it with the ones previously reported. Spiehler and Reed measured blood cocaine concentrations exceeding 20mg/l in three fatalities.<sup>1</sup> Mittleman and Wetli found a blood cocaine concentration as high as 30.9mg/l in their review of 60 cocaine-related overdose deaths.<sup>2</sup> Amon et al. reported a blood concentration of 211mg/l after an ingested bag of cocaine broke in a victim's intestine.<sup>3</sup> The highest blood concentration reported so far in literature was by Peretti et al. of 330mg/l, 1.5 times greater than Amon et al., found in a young woman who had a long history of cocaine abuse.<sup>4</sup>

The last case mentioned jointly with the present one, which records the new highest cocaine blood concentration, offer new insights on human tolerance to cocaine in chronic consumers. Analyzing literature, it seems that nobody but chronic consumers could tolerate doses of cocaine in the order of 10<sup>2</sup>mg/l. To confirm this hypothesis, it would be appropriate for the scientific community to report cocaine intoxication cases as much as possible.

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### Cocaine Overdose, Toxicology, Highest Blood Concentration



## K6 The Identification of Xylazine in a Patient Who Presented for Heroin Withdrawal

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**Learning Overview:** The goal of this presentation is to present a recent case of a patient whose toxicology studies revealed the presence of xylazine and to inform the audience about xylazine as a drug adulterant and drug of abuse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness about a less well-known veterinary medicine and providing information about how to detect the drug in human samples.

**Introduction:** It is important not to overlook veterinary medications as possible drugs of abuse. Having an awareness of these medications is crucial for physicians, forensic pathologists, and toxicologists. Xylazine is a sedative used in veterinary medicine and there are few reports of it being used as a drug of abuse or a drug adulterant in the literature. Detection of xylazine in human samples can be performed by using Gas Chromatography/ Mass Spectrometry (GC/MS).<sup>1</sup> This is a case report of a woman who presented to the emergency department in Royal Oak, MI, for heroin withdrawal, and urine toxicology tests revealed the presence of xylazine.

**Case Presentation:** A thirty-two-year-old female presented to the emergency department with a chief complaint of nausea, intractable vomiting, and tremors. She has a past medical history of Intravenous (IV) drug abuse, alcohol abuse, anxiety, depression, and hepatitis C. Physical exam findings revealed that the patient had tachycardia (heart rate=139bpm) and multiple skin lesions and scars on the upper and lower extremities. A complete blood count revealed that the patient was anemic (hemoglobin=11.8g/dL) and had neutrophilia with a left shift (neutrophils=14.9bil/L). A comprehensive metabolic panel showed an elevated blood glucose (134mg/dL) and an elevated aspartate aminotransferase (45U/L). During the patient interview, she stated that she used heroin the night before she came to the hospital. The patient was admitted to the hospital for heroin withdrawal and was started on IV fluids and antiemetics.

A comprehensive drug analysis was performed and the patient's urine was positive for codeine, morphine, hydromorphone, and 6-acetylmorphine. GC/MS was performed on the urine, which revealed a lidocaine metabolite, nicotine and metabolites, diphenhydramine, xylazine, ondansetron, fentanyl, and acetyl-fentanyl. The patient ultimately left against medical advice two days after coming to the hospital.

**Discussion:** Xylazine is an alpha-2 adrenergic agonist and is used as a sedative, muscle relaxant, and analgesic in veterinary medicine. It is administered to animals intravenously, intramuscularly, and subcutaneously. It has been reported that xylazine has been used on its own as a drug of abuse and as an adulterant for heroin. This medication has also been reported to be used as an adulterant with speedball (a mixture of heroin and cocaine).<sup>2</sup> A study published in *Forensic Science International* in 2014 reviewed the literature and found 43 cases of xylazine intoxication. Out of those 43 cases, 22 of them were fatal.<sup>1</sup>

When xylazine is used alone as a drug of abuse, the main route of administration is injection. However, there have been reports of xylazine being inhaled or taken orally. The physical exam findings for a patient who has overdosed on xylazine include lethargy, miosis, hypotonia, respiratory depression, and hypothermia. The clinical presentation of a patient who has overdosed on xylazine will look similar to a clonidine or opioid overdose. For patients who are intoxicated with xylazine, the treatment plan is supportive. Health care providers need to be aware of the need for endotracheal intubation for respiratory depression.<sup>3</sup> Analytical techniques to identify and quantify xylazine in human samples include GC/MS and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).<sup>1</sup> It's important to note that standard hospital drug screens will not detect the presence of xylazine, like most other Novel Psychoactive Substances (NPSs). The approach that this health system has adopted is designed to be able to confirm the ingestion of NPSs in patients that are still in the emergency room. This has allowed the physicians to attribute certain presenting signs and symptoms to NPSs and treat the patients more appropriately, as well as adding important information regarding local drug surveillance data.

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### Xylazine, Adulterant, Heroin



## K7 An Unusual Case of Suicide by Fluvoxamine Poisoning

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**Learning Overview:** After attending this presentation, attendees will be aware of a particular case of fluvoxamine fatal poisoning.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing blood concentrations of fluvoxamine and other drugs in a fatal intoxication case; these data could be useful in the interpretation of further cases of death.

This case regards a 48-year-old woman with psychiatric disorders, who died in the protected community where she lived. She suffered from obsessive-compulsive disorder, personality disorders, and mild mental retardation.

The patient was treated daily with: haloperidol 2mg/mL (10 drops twice a day); fluvoxamine tablets (150mg); clonazepam (2.5mg/mL; 20 drops three times a day); gabapentin tablets (300mg twice a day); propranolol hydrochloride tablets (40mg); and, when needed, clonazepam (100mg/mL; 20 drops) was also administered.

The woman was found unconscious by the paramedical staff of the protected community at about 3:00 p.m. in the bathroom of her room, where she was heard alive through the closed door at 11:30 a.m. of the same day. After trying to call the woman several times, the health care workers opened the door using a special key. They found the patient sitting on the toilet, her mouth smeared with pink-colored vomit. In her mouth, in her hands, and on the floor there were also many sheets of toilet paper, pink in color. Resuscitation attempts were in vain, and the woman's death was recorded at 3:45 p.m. The appropriate authorities were not notified, and the room was cleaned up and the corpse was prepared for the funeral.

Later, an autopsy was ordered to assess the cause and manner of the death and was performed nine days after the death. External examination was essentially negative with no significant findings, except for some marks caused by resuscitation attempts. Green discoloration of the lower abdomen was present. Pink fluid, with pieces of material similar to the one present in the stomach, were observed in the entire respiratory tract. Smooth material, pink-violet in color, was found in the esophagus. All the viscera were congested. Sporadic subpleural petechiae could be observed. Histological investigations revealed pulmonary edema and the presence of vegetable matter in the bronchioles as well as in the alveoli. In many places, the alveoli were expanded with broken septa.

Blood and urine were collected for toxicological analysis. Quantitative determination of ethanol and systematic toxicological analysis to detect acidic, neutral, and basic drugs was performed on peripheral blood sample. They were conducted using Gas Chromatography/Mass Spectrometry (GC/MS), preceded by a solid phase extraction with mixed-mode cartridges. The analyses allowed the identification of the following drugs: fluvoxamine, clonazepam, 7-aminoclonazepam, propranolol, gabapentin, and haloperidol. Quantification of the detected drugs in the blood was performed by means of a validated Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analytical procedure and the following results were achieved: fluvoxamine (4.3mg/L), gabapentin (41mg/L), 7-aminoclonazepam (0.24mg/L), clonazepam (0.07mg/L), haloperidol (<0.01mg/L), and propranolol (0.24mg/L). Contributory causes of death, due to multiple drugs use, cannot be excluded. However, the fluvoxamine concentration in the blood exceeded approximately 20 times the upper limit of therapeutic blood levels (0.23mg/L) and was consistent with literature data about fatal poisoning.

Fluvoxamine is a Selective Serotonin Reuptake Inhibitor (SSRI) that is used in the management of depression and obsessive-compulsive disorders. Few cases of fluvoxamine intoxication are described in literature, since this drug is considered relatively safe in overdose. Nevertheless, at therapeutic and supratherapeutic concentrations, fluvoxamine might affect cardiac conduction, prolong QTc interval, cause hypotension and obtundation, and increase the risk of seizures.

In the described case, toxicological analyses were essential to determine the cause of death: poisoning with fluvoxamine, complicated by acute mechanical asphyxia due to regurgitated food. The manner of death was deliberate self-poisoning, since further investigations allowed the discovery that the woman probably stole several tablets of fluvoxamine from the clinic.

### Fluvoxamine, Overdose, Postmortem Blood Concentration



## K8 Delta-8-Tetrahydrocannabinol (Delta-8-THC): Increased Prevalence in Drug Seizure Cases and Impact on Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Analysis of Biological Specimens

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**Learning Overview:** After attending this presentation, attendees will understand the increased prevalence of delta-8-THC in drug seizure cases and the potential impact on LC/MS/MS analysis of biological specimens for cannabinoids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing toxicologists of the need to evaluate LC/MS/MS cannabinoid methods for the potential of interference of delta-8-THC with delta-9-THC.

**Introduction:** Delta-8-THC is a cannabinoid that is a minor constituent found naturally in the cannabis plant. Delta-8-THC is an analog of delta-9-THC and has similar effects and receptor activity, but lower potency. Recently, drug seizure exhibits submitted in Palm Beach County, FL, have contained delta-8-THC as the primary constituent or as a mixture with delta-9-THC. The first drug seizure submission was from an incident that occurred in November of 2018. From November 2018 to June 2019, delta-8-THC has been identified in a total of 12 drug seizure cases with 4 cases from May 2019. Ten of the exhibits were vape cartridges and two were food items.

**Objective:** Evaluate the ability of a validated LC/MS/MS blood cannabinoid quantitation method employing a biphenyl LC column to discriminate between delta-8 and delta-9-THC.

**Method:** A previously described LC/MS/MS blood cannabinoid quantitation method that included delta-9-THC, delta-9-hydroxy-THC, and delta-9-carboxy-THC was employed.<sup>1</sup> Briefly, 0.5mL of whole blood was extracted by liquid-liquid extraction with hexane:ethyl acetate (80:20). Analysis was conducted on a Shimadzu® Prominence XR LC system with a SCIEX™ 3200 Qtrap® MS/MS and a Raptor™ Biphenyl column with dimensions of 50 x 2.1mm and a 2.7µm particle size. A gradient LC program was employed with LC/MS water and LC/MS methanol, each with 0.1% formic acid. The calibration range was from 1–40ng/mL for delta-9-THC and delta-9-hydroxy-THC and 5–200ng/mL for delta-9-carboxy-THC. Stable isotope internal standards were used for all three target analytes.

Three different fortified blood samples were prepared and extracted along with the routinely used calibrators and controls to evaluate the selectivity of the method through an interference study: one fortified with delta-8-THC at 40ng/mL without internal standard; one fortified with delta-8-THC at 5ng/mL with internal standard; one fortified with delta-9-THC and delta-9-hydroxy-THC at 5ng/mL, and delta-9-carboxy-THC at 25ng/mL, as well as delta-8-THC at ng/mL with internal standard.

**Results:** There was no interference from a high concentration of delta-8-THC (40ng/mL) with the internal standards. Delta-8-THC did not interfere with delta-9-OH-THC or delta-9-carboxy-THC, but did interfere with delta-9-THC. Delta-8-THC produces the same precursor/product ion transitions in the same relative abundances as delta-9-THC. The retention time for delta-8-THC (4.75) was 0.03 minutes later than the average of the calibrators (4.72), but was still within the 0.1 minute acceptance window. The Multiple Reaction Monitoring (MRM) ratios were slightly higher than the average of the calibrators (36% compared to 32%), but were also within the acceptance range of +/-20%. The 5.0ng/mL delta-8-THC control was identified by the method as containing delta-9-THC at 4.7ng/mL. The mixed control containing both delta-8-THC and delta-9-THC, each at a target concentration of 5.0ng/mL, was identified by the method to contain delta-9-THC at 9.4ng/mL. No split peak or other indication of the presence of two unique compounds was observed. Unpublished reports from laboratories employing C-18 LC columns have observed a split peak with incomplete resolution of delta-8 and delta-9-THC. Drug standards for delta-8-hydroxy-THC and delta-8-carboxy-THC were not available to evaluate potential interferences for the delta-9 counterparts targeted by the method. A polar-C18 column was evaluated and demonstrated the ability to resolve delta-8- and delta-9-THC. Further work will be conducted to develop a method employing a polar-C18 column.

**Conclusion:** The LC/MS/MS blood cannabinoid method employing a biphenyl LC column could not discriminate between delta-8- and delta-9-THC as the compounds have identical MRM transitions and were not resolved chromatographically. Delta-8-THC may also cause interference with delta-9-THC for LC/MS/MS methods that employ a C-18 column. LC/MS/MS methods for the determination of cannabinoids should be evaluated for specificity between delta-8- and delta-9-THC and the potential impact to casework examined.

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### Delta-8-THC, Validation, LC/MS/MS



## K9 The Determination of the Organic Components of Newer Generation E-Cigarette Liquids

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**Learning Overview:** After attending this presentation, attendees will learn how Gas Chromatography coupled to Mass Spectrometry (GC/MS) can be utilized to identify and quantify targeted chemical components in newer generation e-cigarette products. This will include products that contain cannabinoid compounds, such as Cannabidiol (CBD), in addition to nicotine and other materials.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing analysts with a relatively simple method to analyze e-cigarette products and informing them about the potential harms the constituents of e-cigarette liquids may pose to the public without proper product regulation and quality control measures.

In 2003, the electronic cigarette (also known as an e-cigarette, electronic nicotine delivery system or ENDS, vaporizer device, vape pen, etc.) was introduced to the global market, reaching the United States in 2007, and quickly growing into an extremely lucrative enterprise.<sup>1,2</sup> E-cigarettes differ from traditional cigarettes through the use of a heating element/atomizer to heat a cartridge composed of purified and processed nicotine, propylene glycol, vegetable glycerin, additives, and flavorings to the point of vaporization.<sup>2-7</sup> This vapor is then inhaled by the user. It is hypothesized that e-cigarettes have become such a successful product due to being heavily marketed as a smoking cessation aid and as a “healthy” alternative to traditional cigarettes.<sup>2,5,6,8,9</sup> Additionally, companies are using more modern market avenues to advertise and sell their products and are offering an extremely wide variety of nicotine concentrations and flavors that are not available for cigarettes and other nicotine alternatives.<sup>2,5,6,8,9</sup> E-cigarette vaporizers range from the first generation “cig-alike” devices to highly customizable third generation “mods” to the more recent and well known JUUL™.<sup>5</sup>

Recently, e-cigarettes have been focused on by the media with claims of lack of regulation by the Food and Drug Administration (FDA) and poor quality control.<sup>3,8-10</sup> The absence of these measures has resulted in inconsistent policies at the local, state, and federal levels and a lack of knowledge about the substances present in e-liquid and the subsequent produced vapor.<sup>6</sup> However, the largest issue addressed includes accusations that the introduction of e-cigarettes has sparked an epidemic of nicotine addiction among American youth that may act as a gateway for non-smokers to transition to traditional smoking or more illicit drug use.<sup>1,2,5,6,9,11-13</sup> The goal of this project is to further investigate and possibly identify the potentially harmful chemical components either present, or created through the use of, newer e-cigarette products common among young people in the United States.

Different types of e-cigarette liquid and devices were investigated in this project, including bulk e-liquids with a SMOK® MAG Kit third-generation device or “mod”, JUUL™ and JUUL™-compatible cartridges (this includes JUUL™, SEA100™, and Hempzilla™ pods) with a JUUL™ e-cigarette device, and thicker CBD oils and/or cannabis e-liquid cartridges with a Yocan® Evolve 2.0 Vape Pen. Samples were prepared by attaching the vaporizer devices to a smoking pump to produce vapor for each e-liquid. This vapor was pulled through a water trap in a glass impinger in order for its components to be trapped in the water and separated from the viscous propylene glycol and vegetable glycerin found in e-liquids, which are not very compatible with GC/MS systems. A liquid-liquid extraction was then performed with dichloromethane, and the samples were analyzed with an Agilent® 7890A GC and a 5975C inert MSD with Triple Axis Detector. The column utilized for separation was an Rtx-5 column (Restek®, 30m, 0.25mm ID, 0.25µm film thickness). Targeted sample components were identified using the National Institute of Standards and Technology (NIST) 2014 database and comparison to certified reference materials. Identified components were quantified via calibration standards for each target. Substances of interest included nicotine, formaldehyde, acetaldehyde, diacetyl, acetyl propionyl, CBD, THC, and others. This selection was based on previous research regarding the harmful components found in traditional cigarettes and potentially in e-cigarette products.

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### **E-Cigarette, Gas Chromatography, Drug Chemistry**



## K10 Driving Under the Influence of 1,1-Difluoroethane (DFE) and Marijuana

Aybike Dip, PhD, Turkish Ministry of Justice, Adana 00100, TURKEY; Ashraf Mozayani, PharmD, PhD\*, Texas Southern University, Houston, TX 77004

**Learning Overview:** After attending this presentation, attendees will better realize the intoxicating effects of DFE and marijuana on driving performance.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of DFE abuse and the importance of immediately collecting blood samples from drivers in traffic accidents. The knowledge gained will help improve investigations into vehicle accidents involving DFE and/or marijuana consumption.

DFE is a colorless, flammable hydrofluorocarbon with a slight ethereal odor. It is widely used in refrigeration, electronic cleaning products, and various aerosol propellant products that are sold in many markets.<sup>1</sup> When DFE is inhaled or huffed, it causes short but intense euphoria.<sup>2</sup> Due to its euphoric effects, it is abused by people who can easily access them, even in local supermarkets. The high from DFE inhalation can cause dizziness, euphoria, hallucinations, disorientation, loss of motor control, confusion, memory loss, pulmonary irritation, loss of consciousness, and sudden death by causing heart failure, even on the first use. Investigations of driving incidents involving DFE have shown that drivers who lost consciousness frequently have no memory of events immediately prior to the accident.

This case involved a 20-year-old man who went for a ride with his friends early in the morning. While driving around the city, he stopped the car at a local supermarket, where one of the passengers stole a can of air duster that contained compressed air used to clean computer keyboards. After leaving the market, he huffed some of the mixture from the can and passed it to the driver. A few minutes after huffing, the driver lost control of the vehicle, crashed into and snapped off a wood telephone pole, then proceeded to crash into a store. There were no fatalities; however, the driver as well as all passengers were severely injured due to the accident.

A blood sample of the driver was taken about an hour after arrival at the hospital. Blood test results were found as 0.92ng/mL of delta-9-THC (which is active ingredient of marijuana), 16ng/mL of delta-9-carboxy-THC by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), and 2µg/ml DFE by Gas Chromatography/Mass Spectrometry (GC/MS). No alcohol was detected. Per research, this appears to be the first case report of a traffic accident that involved both DFE and marijuana consumption of a living subject.

It can be concluded that the DFE level was consistent with other Driving While Intoxicated (DWI) incidents and the presence of both DFE and THC in the blood were contributing factors to the accident.

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### 1,1-Difluoroethane, Marijuana, DUI



## K11 Driving Under the Influence (DUI) in the United States and Brazil

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**Learning Overview:** The focus of this research is a comparative investigation of the forensic science and criminal justice aspects of DUI between the United States and Brazil.

**Impact on the Forensic Science Community:** With globalization, it is essential to know the practical realities within legal traditions and forensic science in different countries. This presentation will impact the forensic science community by increasing familiarization of similarities and differences between two countries in the application of forensic science and the law.

This study focused on comparisons of institutional structures, laws, and forensic evaluations. This study performed literature-based research from several databases: institutional information, gray literature, jurisprudence, and other enlightening sources. The comparisons were mainly overextending of forensic evidence, law definitions included the category of offenses, types, and limits of each drug, classes of tests, and other weighty information. Regarding institutional aspects, in the United States, DUI is mainly a state law offense. A federal DUI charge arises for crimes that occur on federally owned land or another location that is considered federal property. In Brazil, there is a single Code, Law 9.305/1997, which is applied to the entire country with no difference among the states.

In respect to variations in law, in the United States, some states' laws differentiate between the terms DUI and Driving While Intoxicated (DWI), but in most, the terms are interchangeable. In Brazil, there is no difference between DUI and DWI. For all states in the United States, a person is deemed to be intoxicated when the Blood Alcohol Concentration (BAC) is equal to or above 0.08% (0.08g dL<sup>-1</sup>). The penalties are also different for each state. In Brazil, there are the following situations: (1) Article 165 of the Brazilian Traffic Code considers as a misdemeanor driving under the influence of alcohol. Any amount of alcohol detected in the breathalyzer subjects the driver to the penalties foreseen in Article 165—a fine and a suspended license for 12 months, and (2) Article 306 establishes as a crime a BAC above 0.6g L<sup>-1</sup> or 0.3mg L<sup>-1</sup> exhaled air, and the punishment involves a prison sentence. In the United States, the extension of drug testing is different in each state and even in a different part of one state. Sometimes no drug gets tested if the level of alcohol at the time of driving was more than 0.08 mg L<sup>-1</sup>. In Brazil, the Nacional Transit Council (Conselho Nacional De Trânsito (CONTRAN)) states that some tests shall be performed to confirm the alteration in psychomotor ability. The influence of alcohol can be verified through a blood test or regulated breathalyzers. Other laboratory examinations will confirm the influence of other psychoactive substances. The transit agencies or the judicial police are responsible for indicating the laboratories permitted to perform the exams (Resolution 432/2013). The laws of both countries also provide punishment consequences for a refusal to take a breathalyzer test. A comparison of forensic aspects revealed that, although there are significant differences between the United States and Brazil regarding the types and amounts of substances and the relative penalties, there are also similarities such as: (1) chemical tests related to detecting the type and quantity of the substances; and (2) the police officer's testimony about the behavior of the driver is considered as evidence for DUI.

In conclusion, comparative research can be useful for disseminating the laws of other countries, which reflects differences in cultures, history, and societies between countries, in this case, between the United States and Brazil.

### Driving Under Influence, Comparative Research, United States and Brazil



## K12 Methadone-Related Deaths: A Six-Year Study in a Major Italian City

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**Learning Overview:** After attending this presentation, attendees will learn about the risks of self-administration of methadone to counter heroin abuse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the relevance of carrying out a complete autopsy examination, supported by histological and toxicological evaluations, in patients with methadone therapy, in order to identify the real cause of death and the contemporary consumption of other drugs. This presentation will also impact the forensic science community by underlying the implication of the increase in illegal sales of substances for self-administration together with methadone as a substitutive therapy and related risks in non-addicted patients.

Methadone is a synthetic opioid, a pure agonist of the  $\mu$  receptor. It has the advantages of high oral bioavailability, long half-life, absence of active metabolites, and low cost. Like other opioids, methadone causes addiction and tolerance. Side effects of methadone are pulmonary edema and respiratory depression, particularly in the chronic abuser, and ventricular arrhythmia in those subjects who consume it in high doses for illicit use. In Italy, methadone is commonly distributed by the National Health System for heroin substitutive therapy in addicted patients. Dedicated structures provide methadone for these patients. In the past years, national studies of incidence and prevalence have demonstrated an increase in illegal sales of methadone and, consequently, also an increase in deaths due to acute methadone intoxication.

This study included 31 subjects' deaths due to methadone consumption: 25 were male and 6 females. Eleven subjects (36%) were under substitutive treatment at the time of death, 3 subjects were previously monitored, and 11 subjects were unknown to the addiction therapy system. In all cases, a complete autopsy was performed and blood samples were collected for toxicological analysis using gas chromatography/mass spectrometry.

The concentration range of methadone in the blood samples was 46 to 4,058.53ng/mL, with an average of 821.46ng/mL. The range in patients under substitutive treatment was 61.8 to 4,058.53ng/mL, with an average of 1,148.86 ng/mL. The range in other subjects was 46 to 1,830ng/mL, with an average of 614.68 ng/mL. Five subjects presented a single positivity to methadone (three were followed by the National Health System, with an average concentration of 2,028.51ng/mL and the other two with an average methadone concentration of 1,166.5ng/mL). Other subjects were positive for different drugs or substances in sub-lethal concentrations: 13 subjects with alcohol, 11 with cocaine, 7 with benzodiazepines, 5 with other opioids, 2 with tetrahydrocannabinol, 3 with neuroleptics, 1 subject with serotonin re-uptake inhibitors and 1 with barbiturates; 84% of patients were positive for at least two substances.

Such results of methadone concentrations correspond to known lethal dosages. According to the literature, usual consumers of methadone develop a much higher tolerance than subjects who take it for illicit use.

The substitutive therapy with methadone is effective and works properly, but illegal sales and consumption of methadone, which has increased in recent years, can have a negative impact on self-administration of opioid addiction treatment therapies, inducing patients who enhance their own doses; moreover, it produced a rise in methadone-related deaths. Accordingly, careful monitoring of doses administered to patients is requested to make the system safer. In this context, a systematic urinary research of sucrose and lactose (disaccharides), in order to discover—with a high degree of reliability—the route of administration, could represent a medicolegal contribution to contrast and identify illegal intravenous methadone intake and related deaths.

### Methadone, Forensic Toxicology, Drug Addiction



## K13 Comparison of Ethanol Concentrations in Blood and End-Expired Breath During a Controlled Drinking Study

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**Learning Overview:** After attending this presentation, attendees will understand how near-simultaneous Blood Alcohol Concentration (BAC) and Breath Alcohol Concentration (BrAC) quantification compares as ethanol is absorbed and metabolized during a controlled drinking study.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by comparing two popular methods of quantifying bodily ethanol concentration.

Ethanol consumption results in diverse outcomes, such as: intoxication, cognitive impairment, motor incoordination, and dependence.<sup>1</sup> These effects are likely due to ethanol's action on multiple brain proteins, specifically GABA<sub>A</sub> receptors in the central nervous system.<sup>2</sup> Although ethanol's mechanism of action in the brain has yet to be fully understood, ethanol's impairing effects are well documented and bodily alcohol concentration per se limits have been incorporated into various laws. BAC and end-expired BrAC are two popular surrogates to quantify ethanol impairment via ethanol concentration in the brain and are often cited in per se statutes. This study seeks to compare BAC (g/100ml) and end-expired BrAC (g/210L) techniques and results as performed in Wisconsin.

Measurements of ethanol concentration were determined from blood and end-expired breath during a controlled drinking study in which healthy men and women drank 0.55–1.04g ethanol per kg body weight in 60 minutes. Specimens of breath were obtained for analysis of ethanol starting at 30 minutes post-dosing, then every 15-20 minutes for two hours to obtain a curve. One of the BrAC samples in the series was accompanied by a BAC sample to determine measurement agreement throughout the curve. BAC was determined by headspace gas chromatography and BrAC was determined with an electrochemical fuel cell (Intoximeter's EC/IR II), reporting the lower of two correlating breath samples. Results of the two subject breath samples required in Wisconsin's evidential breath test sequence will be discussed as they were highly correlated ( $r=0.98$ ). Additionally, breath alcohol curves will be presented with corresponding BACs, which were also highly correlated ( $r=0.94$ ). Furthermore, comparison of measurement techniques revealed that BrAC results were generally less than the corresponding BAC by 11.8%.

When quantifying and discussing ethanol impairment, it is important to recall that both BAC and BrAC measurements are surrogates for the ethanol concentration in the brain. Results of this study demonstrate how sampling, measurement, and reporting variables impact the final ethanol concentration assessment. Both BAC and BrAC measurements are excellent proxies for ethanol concentration in the brain, and associated impairment at each concentration is well defined in other literature.<sup>3</sup> However, details of the measurement technique and any legally mandated reporting requirements should be considered and explicitly defined when describing ethanol concentration results, particularly while referencing per se limits.

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### Impaired Driving, Breath Alcohol Concentration, Blood Alcohol Concentration

## K14 Longitudinal Transdermal Fentanyl Compared With Morphine Sulfate Treatments in a Rabbit (*Oryctolagus cuniculus*) Model System: Impacts on Behavior and Health

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**Learning Overview:** The goals of this presentation are to: (1) describe the longitudinal effects of systemic opioids, particularly fentanyl and morphine sulfate, on behavioral and physiologic parameters; and (2) demonstrate the long-term use of transdermal fentanyl patches in the management of analgesia in a rabbit model system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing novel longitudinal data regarding the behavior and physiologic effects of prolonged opioid use in a rabbit model system. With the current trend of opioid overdoses poised to expand its impact further, forensic scientists will learn an empirical methodology for evaluating soft and hard tissue responses, behavioral changes, and physiological effects associated with prolonged opioid use in a live animal proxy.

Transdermal fentanyl patch systems have been applied to various animal species, including dogs, cats, swine, rabbits, and non-human primates.<sup>1-6</sup> However, in rabbits, the transdermal fentanyl patch literature remains sparse and its application has been investigated in a segmented manner.<sup>4,5</sup> Given the limited data available, an opportunity exists to improve understandings of the longitudinal effects of opioids on rabbit behavior and physiology. This is critical given that rabbits remain a popular laboratory animal for research activities. For example, in 2017, more than 54,000 rabbits were reportedly involved in animal research involving pain and received a pain-relieving drug as a result. Thus, the primary purpose of the current research was to develop a longitudinal model for studying the effects of prolonged opioid exposure, specifically fentanyl and morphine sulfate, in a rabbit model system.

Skeletally mature, 6-month old (2.3-3.0kg), male New Zealand White rabbits (*Oryctolagus cuniculus*;  $n=21$ ) were sourced from Covance Research Products Inc. Rabbits were individually housed in stainless steel rabbit batteries that allowed for some interaction, while keeping the animals lodged separately. The rabbits were quarantined and habituated to the testing conditions for a two-week period. Animals were randomly divided into three groups of seven animals each: morphine, fentanyl, and controls. The control group was further randomly divided into saline vehicle ( $n=3$ ) and transdermal patch groups ( $n=4$ ). Following the acclimation period, the experimental treatments for the opioid groups (morphine and fentanyl) were initiated and continued for eight weeks. The morphine sulfate group received a dose of 3mg/kg/day via subcutaneous injection. The control group was administered saline at a dose of 3mg/kg/day. A 25- $\mu$ g/h slow release transdermal fentanyl patch was placed on the interscapular region of rabbits in the fentanyl group every third day, following shaving of the hair. Patches were secured by an overlying Tegaderm™ transparent film dressing. A placebo patch (Tegaderm™) was placed on each control patch animal comparable to the experimental animals. Rabbit jackets from Lomir Biomedical were applied to all experimental patch (fentanyl) and control animals (Tegaderm™) in order to prevent the animals from chewing the dressings.

Daily observations were recorded at consistent time points. Classifications for fecal output and food consumption were assigned values of 1 (normal), 2 (moderate), and 3 (low), and compared among the treatment groups and controls using one-way Analysis of Covariance (ANCOVA) tests with jacketing as a co-variate. Fecal output was not significantly impacted by drug treatment ( $F_{(2, 17)}=0.286$ ,  $p=0.755$ ,  $\eta^2=0.033$ , power=0.095). Food consumption was significantly impacted ( $F_{(2, 17)}=5.44$ ,  $p=0.015$ ,  $\eta^2=0.390$ , power=0.841), and a Tukey's Honest Significant Difference (HSD) post-hoc test indicated that morphine rabbits were significantly depressed in food consumption compared to controls ( $p=0.012$ ).

Qualitatively, all fentanyl patch rabbits consistently displayed skin irritation and erythema, abnormal stress behaviors (e.g., acute anorexia), respiratory depression, ocular proptosis, and poor overall health compared to the morphine and control groups. While the morphine injections did not result in notable changes in outward appearance, these animals demonstrated consistently lower food consumption throughout the experiment and heavy sedation following treatments. Despite its prevalence in short-term studies in the literature, these findings suggest that long-term experimental use of transdermal fentanyl patches results in behavioral changes that may confound the effects caused solely by the narcotic in question.

This report is the first documented data characterizing longitudinal transdermal fentanyl patches as a route for administration of analgesia in rabbits. The documented adverse effects associated with such longitudinal treatments addresses an evident literature gap that will inform future forensic, biomedical, and veterinary medicine research.

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### Fentanyl, Rabbits, Morphine

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## K15 Fatal Formalin Intoxication in Thailand: A Case Report

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**Learning Overview:** After attending this presentation, attendees will be able to obtain practical information from a case of acute formalin poisoning in terms of clinical presentation, autopsy, and histological findings and toxicological results.

**Impact on the Forensic Science Community:** This study will impact the forensic science community by expanding knowledge of acute formalin poisoning, which is relatively rare in medicolegal cases, and by promoting safety awareness about keeping hazardous chemicals in a hospital environment.

Formalin, an aqueous solution of formaldehyde, is a common solvent used for medical purposes. It is frequently used for tissue fixation in pathology and in dead body preservation as embalming fluid. In Thailand, the embalming procedure is commonly performed by hospital personnel, for example, autopsy staff. In general, embalming fluid in Thailand frequently contains formalin, methanol, phenol, glycerin, buffers, and nitrate compounds. As embalming fluid or formalin has a pungent odor and highly irritating effects, suicidal or accidental ingestion of embalming fluid or formalin is relatively uncommon in medicolegal cases.

This study reports a case of acute and fatal formalin poisoning in a 24-year-old male who was a patient transport staff person in the hospital. The patient drank clear liquid in a water bottle placed in the hospital locker, which was later found to contain chemicals used for embalming fluid preparation. He presented with nausea and vomiting, abdominal pain, and alteration of consciousness. He was then sent to the emergency department. His laboratory results showed severe wide anion gap metabolic acidosis, hypocalcemia, and hypokalemia. His Electrocardiogram (EKG) displayed diffuse ST depression in limb leads and chest leads. He then developed hypotension, Disseminated Intravascular Coagulation (DIC), and upper gastrointestinal hemorrhage. After seven hours in the hospital, he was pronounced dead after unsuccessful cardiopulmonary resuscitation. Due to the source of water found in the bottle he drank and his clinical manifestation, his provisional diagnosis by the attending physician was acute formalin toxicity. After the postmortem inquest, his body was sent for a medicolegal autopsy at the Department of Forensic Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University for the investigation of cause of death.

Autopsy findings revealed grayish-black discoloration of both tonsils, right palatopharyngeal arch, and some areas of tracheal mucosa. In addition, lower esophageal mucosa was mildly inflamed and eroded, whereas gastric mucosa was transformed into a leathery, hardened, and grayish-black color at fundus and body of greater curvature and gastric mucosa at lesser curvature was markedly edematous. Histological findings showed necrosis of gastric mucosa and acute tubular necrosis of the kidney. Formaldehyde was detected in a postmortem femoral blood sample, gastric content, and the water bottle from which he drank. The review of previous studies in formalin or formaldehyde ingestion is also presented for comparison to this study. It is found that autopsy and toxicological findings in this case are consistent with the results from previous studies, particularly in wide anion gap metabolic acidosis and caustic injury to the stomach greater than esophagus. This result provides additional useful information for interpretation of acute formalin toxicity.

### Formalin, Intoxication, Autopsy



## K16 Determination of Chiral Cathinone in Fresh Samples of *Catha Edulis*

Abdulrhman M. Dhabbah, PhD\*, King Fahad Security College, Riyadh, SAUDI ARABIA

**Learning Overview:** The goals of this presentation are to show: (1) how using Menthyl Chloroformate (MCF) to derivatize cathinone enantiomers and yield two diastereomers; (2) the quantitative determination of *S* and *R* cathinone confirmed the presence of both enantiomers in all parts of the studied plant; (3) the screening of active constituents in different parts of the plant; and (4) the comparison of characteristic distribution between *S* and *R* cathinone in leaves and stems in Khat.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating: (1) the quantitative determination of *S* and *R* cathinone confirmed the presence of both enantiomers in all parts of the studied plant; (2) the concentration of *S*-cathinone was higher in stems while its values were lower in leaves; (3) the highest content of *S*-cathinone, which is the most psychoactive stereoisomer, was measured in the upper stems of the plant; and (4) the present study is the first quantitative investigation of the two cathinone enantiomers in different parts of fresh *Catha edulis*.

*Catha edulis* Forsk, commonly named Khat, is an evergreen wild shrub cultivated in East Africa and the Arabian Peninsula. It is traditionally consumed in these regions for its psychoactive properties. The main stimulating compound in Khat is cathinone, which consists of two enantiomers; several studies have established that *S*-(-)-cathinone is more stimulant than its *R* isomer. However, the quantitative determination of *R* and *S* cathinone in *Catha edulis* was not mentioned in previous papers. Moreover, there is still controversy about the presence of the *R* enantiomer in this plant. Thus, the present work aimed to determine the two cathinone enantiomers in different parts of fresh Khat samples.

Extraction of cathinone was carried out from different parts of fresh *Catha edulis*. The derivatization of cathinone enantiomers was done using MCF and yielded two diastereomers, which were separated by gas chromatography on a HP-5 capillary column with an excellent resolution factor (*R*s higher than 5). Figure 1 shows the chromatogram of a leaf extract after cathinone was derivatized with MCF. The three main peaks observed at 20.938, 31.857, and 33.887min. correspond to MCF, *S*-cathinone and *R*-cathinone derivatives, respectively. The structure of the two cathinone diastereomers was confirmed by mass spectrometry. Their mass spectra are shown in Figure 2; the same main fragments are observed in both spectra at *m/z* 83, 226, 105, 182, and 139, but with different abundances.

The quantitative measurements were based on a calibration curve obtained by injection of a series of standard solutions of *S*-(-)-cathinone in the concentration range from 1 to 100µg/mL. The quantitative results of *S* and *R* cathinone confirmed the presence of both enantiomers in all parts of the studied plant, while some previous studies mentioned that only *S*-cathinone was detected in this species. Also, the obtained results showed a characteristic distribution in the different parts of the plant. The concentration of *S*-cathinone was higher in stems while its values were lower in leaves. The concentrations were in the ranges 0.081-0.290mg/g and 0.087-0.211 mg/g for *S* and *R* isomers, respectively. On the other hand, the highest content of *S*-cathinone, which is the most psychoactive stereoisomer, was measured in the upper stems of the plant. The characteristic distribution of *S* and *R* cathinone was illustrated by their enantiomeric excess (ee%) in the different portions of the branch. The ee% values were positive in all stem samples of Khat, while in contrast they were negative in leaf samples. These results confirm the predominance of the most psychoactive *S*-cathinone in stems. The present study is the first quantitative investigation of the two cathinone enantiomers in different parts of fresh *Catha edulis*.

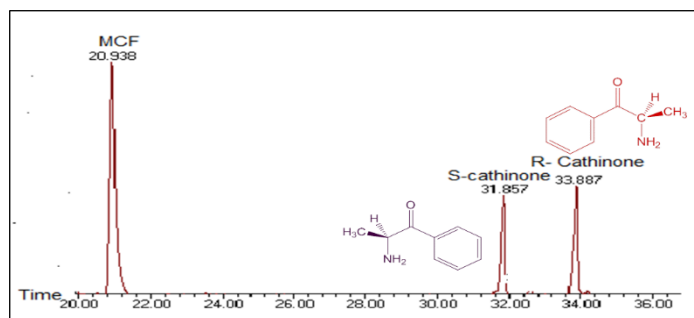


Figure 1: Chromatogram of leaf extract after derivatization with MCF

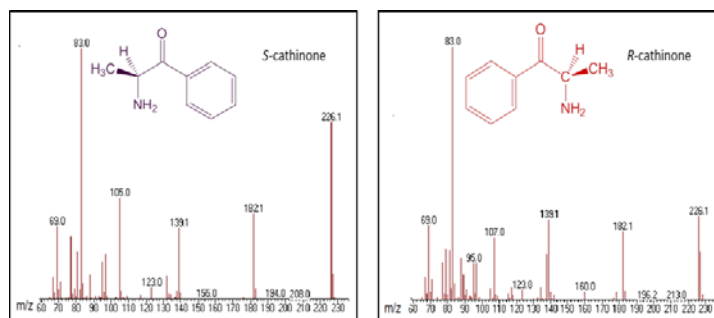


Figure 2: Mass spectra of cathinone diastereomers

The present study is the first quantitative investigation of the two cathinone enantiomers in different parts of fresh *Catha edulis*. The quantitative results of *S* and *R* cathinone confirmed the presence of both enantiomers in all parts of the studied plant, while some previous studies mentioned that only the most psychoactive stereoisomer *S*-cathinone was detected in this species. The concentration of *S*-cathinone was higher in stems while its values were lower in leaves. On the other hand, the highest content of *S*-cathinone was measured in the upper stems of the plant.

### S/R-Cathinone, Derivatization, GC/MS



## **K17 A Targeted Qualitative Screen for the Detection of Pesticides in Postmortem Specimens by Ultra High Performance Liquid Chromatography-Ion Trap-Mass Spectrometry (UHPLC-Ion Trap-MS<sup>n</sup>)**

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**Learning Overview:** After attending this presentation, attendees will better understand the qualitative identification of 13 pesticides in postmortem samples by UHPLC-Ion Trap-MS<sup>n</sup>. Pesticide classes included in the study were common organophosphates, carbamates, triazines, and triazoles. The majority of the analytes used in this method were pesticides found at a local hardware store or used in the agricultural industry specifically for the treatment of insects, weeds, or fungi found on tropical plants or fruits.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a validated targeted screening method for the qualitative identification of pesticides in postmortem specimens and how to apply the method to several medical examiner cases in which a pesticide is suspected as a cause of death.

In Miami-Dade County, exposure to harmful pesticides is common due to its location, tropical climate, and large agricultural industry. Forms of exposure can include overspray from airplanes or tractors, occupational (farmers and landscapers), or household (improper use, storage, and application). From 2014 to 2017, Florida Poison Information Center Network ranked pesticides among the top ten exposure categories for Miami-Dade County with approximately 400 calls related to pesticide exposure reported annually. Although the number is underreported, the Miami-Dade Medical Examiner Department (MDME) reported approximately 1-2 cases per year since 2004 in which a pesticide was listed in the cause of death or in which pesticide poisoning was suspected in the cause of death. Due to the prevalence of pesticide use in the community and the potential for accidental or intentional fatalities, it is imperative that laboratories are able to identify pesticides in postmortem matrices.

The method validation plan included the evaluation of Limit Of Detection (LOD), carryover, interferences, and autosampler stability. Blank blood was utilized for the method validation studies, whereas medical examiner casework analysis included the evaluation of blood, serum, urine, and/or gastric contents obtained at autopsy. Then 500µL of specimen was fortified with a deuterated internal standard solution containing atrazine-D<sub>5</sub>. Protein precipitation was performed by the addition of 2mL of acetonitrile:methanol (95:5), followed by centrifugation at 2,800g for 10 minutes. The supernatant was analyzed using a Thermo Scientific™ UltiMate™ 3000 UHPLC coupled to a Bruker® amaZon™ SL Ion Trap Mass Spectrometer. Chromatographic separation was achieved with a Thermo Scientific™ Acclaim® RSLC 120 C18 column (2.1 x 100mm, 2.2µm) at 40°C with an aqueous mobile phase consisting of 2mM ammonium formate, 0.1% formic acid, and 1% acetonitrile in water, and an organic mobile phase consisting of 2mM ammonium formate, 0.1% formic acid, and 1% water in acetonitrile. Positive electrospray ionization was utilized with a scan range of 50-450 *m/z*. Tandem Mass Spectrometry (MS/MS) data analysis included multi-step identification using a data-dependent scheduled precursor list, which utilizes retention time and molecular ion data to trigger MS<sup>n</sup> analysis.

An in-house library containing 13 pesticides of interest and an internal standard, including retention time, parent ion spectra, and daughter ion spectra, was created based on the analysis of neat PESTANAL® standards. Chromatographic separation of all pesticides was achieved within the first ten minutes. The experimental LODs ranged from 0.10mg/L to 1.0mg/L. Carryover was not observed for any pesticide up to 10mg/L, except for diazinon, which was free from carryover up to 5mg/L. No exogenous interferences were observed. Extracts were stable on the autosampler for all pesticides up to 72 hours.

Although forensic toxicology laboratories largely deal with illicit substances, over-the-counter medications, and prescription medications, everyday household chemicals, such as pesticides, are just as fatal. With the ease of availability of pesticides to the general public, it is no surprise that they are the result of accidental or intentional poisonings and/or fatalities. Symptoms of pesticide toxicity vary depending on the pesticide, but many include nausea, vomiting, headache, dizziness, abdominal pain, and central nervous system depression; which are easy to mistake for common ailments. The development of this assay demonstrated that protein precipitation followed by UHPLC-Ion Trap-MS<sup>n</sup> analysis was a suitable method for the detection of pesticides in biological matrices. The combination of a qualitative targeted screen for pesticides, pathological findings, and clinical manifestations can be used to aid the medical examiner in determining cause and manner of death in cases where pesticide toxicity is suspected.

### **Pesticides, Postmortem Forensic Toxicology, UHPLC-Ion Trap MS/MS**



### K18 Application of Biochip Array Technology to the Simultaneous Screening of Drugs From a Single Hair Sample Using the Biochip Analyzer Evidence Investigator

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**Learning Overview:** After attending this presentation, attendees will gain an understanding about the application of biochip array technology to the simultaneous screening of drugs in hair using a semi-automated biochip analyzer. This application increases the screening capacity during the drug testing process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing results from the evaluation of this new application that allows simultaneous screening of multiple drugs: amphetamine, benzodiazepines, benzoylecgonine/cocaine, cannabinoids, hydrocodone, ketamine, methamphetamine, opiates, oxycodone, and phencyclidine from a single hair sample. Simultaneous immunoassays arrayed on the biochip surface allow this multi-analytical approach, which leads to test consolidation and an increase in the screening capacity in test settings.

Biochip array technology enables the simultaneous detection of multiple analytes from a single sample. The collection of hair samples is non-invasive and the use of this matrix for drug testing provides a large window of detection as well as the history of drug exposure over time for an individual. Society of Hair Testing (SoHT) and European Workplace Drug Testing Society (EWDTs) guidelines for drug testing in hair are in place.

Competitive chemiluminescent biochip-based immunoassays were employed. Ligands were immobilized and stabilized onto the biochip surface defining an array of discrete test sites. The signal output was inversely proportional to the concentration of drug in the sample. The immunoassays were applied to the biochip analyzer Evidence Investigator. With this system, 54 biochips can be handled at a time. The extraction of the drugs from hair samples (50mg) involved water addition, centrifugation, and the addition of methanol and zircon pulverizing beads. Following spinning and pulverization, methanol was added; after decantation and drying, the reconstituted sample was added to the biochip. The total assay time including wash, extraction, and assay, was 12 hours.

The assays presented the following cut-off and Limit Of Detection (LOD) values respectively: 0.04ng/mg and 0.032ng/mg for amphetamine, 0.02ng/mg and 0.004ng/mg for benzodiazepines, 0.2ng/mg and 0.018ng/mg for benzoylecgonine/cocaine, 0.01ng/mg and 0.008ng/mg for  $\Delta^9$ -THC, 0.001ng/mg and 0.0008ng/mg for THC-COOH, 0.04ng/mg and 0.01ng/mg for hydrocodone, 0.5ng/mg and 0.354ng/mg for ketamine, 0.15ng/mg and 0.06 ng/mg for methamphetamine, 0.04ng/mg and 0.02ng/mg for opiates, 0.1ng/mg and 0.046ng/mg for oxycodone, and 0.02ng/mg and 0.01ng/mg for phencyclidine. Inter-assay precision expressed as percentage Coefficient of Variation (CV) ( $n=15$ , 50% cut-off, cut-off and +50% cut-off), was <18% for all the assays. Authentic hair samples ( $n=43$ ) were assessed with the biochip-based technology and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), the percentage agreement was 98% (amphetamine, benzoylecgonine/cocaine), 95% (cannabinoids, opiates), and 86% (methamphetamine).

In conclusion, the results indicate applicability of biochip array technology to the simultaneous screening of drugs from a single hair sample. Regarding the cut-offs, for drugs appearing in the SoHT or EWDTs guidelines, the cut-off for ketamine was the same and for amphetamine, benzodiazepines, benzoylecgonine/cocaine,  $\Delta^9$ -THC, methamphetamine, and opiates, the cut-offs were lower with the biochip platform, reflecting high sensitivity. Favorable agreement with LC/MS/MS was found for the drugs present in the authentic samples. With the Evidence Investigator, multiple samples can be assessed at a time (up to 54 biochips can be handled at the same time,) which further increases the screening capacity.

#### Biochip Array, Multi-Drug Testing, Hair Testing

## K19 Driving Under the Influence of Alcohol (DUI) and Drugs (DUID) in Southern Italy: Case Reports Showing the Necessity of a Multidisciplinary Protocol to Prove DUI and DUID

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**Learning Overview:** After attending this presentation, attendees will better understand the need to standardize laboratory protocols for alcohol and drugs analysis on blood and urine specimens, as well as protocols for police, physicians, and first aid doctors to verify whether drivers involved in road accidents are impaired.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how only synergistic cooperation among police, physicians and first aid doctors, forensic toxicologists, and forensic engineers allows for solving the question regarding driving impairment.

Driving impairment is not easy to assess because of a variety of factors, which include prior exposure to drugs, degree of both acute and chronic tolerance, innate physical condition, and associated limitations.<sup>1</sup> Studies regarding toxicological findings among drivers hospitalized after a crash are still insufficient and not consistent either in sampling or in the analytical approach. Moreover, in Italy, toxicological analyses and impairment studies after car crashes are not routinely performed by hospital personnel but must be requested by a public prosecutor.<sup>2</sup> The most frequently used protocols in the clinical laboratory are urine immunoassay drug screenings and serum alcohol enzymatic tests. Generally, biological samples analysed are not preserved.

Reported here are three emblematic cases for discussion in which the prosecutor's office ordered the seizure of blood and urine samples taken from possibly impaired drivers and asked the court for forensic toxicological analyses.

A 20-year-old male crashed frontally with another car: only A survived. Toxicological analyses showed the presence of alcohol in blood and urine, respectively 2.01 and 2.13g/l. The biological samples were taken 51min after the car accident. The judge charged A with voluntary murder, regardless of the possible intoxication from alcohol, because the officers present at the crime scene declared A was able to understand and was not impaired.

A 37-year-old man hit a motorcycle with his car and killed the biker. The toxicological analyses showed the presence of morphine (0.23ug/l), 6-MAM (0.04µg/l), and Δ9THC (0.104 µg/l) in the blood and the presence of morphine (>1.00µg/l) and Δ9THC-COOH (>0.16 µg/l) in the urine. The biological samples were taken 1 hour and 57 minutes after the car accident. A forensic engineer showed that D had started to brake too late, with a delay of 2 seconds after the impact. The judge charged D with road murder aggravated by impairment (related to the presence of psychoactive substances in blood and to the engineer statement), even though the paramedic declared D was able to understand and was not impaired.

B, a 40-year-old man, lost control of his car and violently crashed into the car of F, who died. The toxicological analyses showed the presence of 7-aminoclonazepam (74ng/ml), methadone, and 2-Ethylidene-1,5-Dimethyl-3,3-Diphenylpyrrolidine (EDDP) (respectively 5,386ng/ml and 5,648ng/ml) in the urine. A blood sample, negative for alcohol, was thrown away. The judge charged B with road murder, but he was not declared drug impaired, even though the policeman stated he was "definitely confused."

In conclusion, only a synergistic cooperation between police, physicians and paramedics, forensic toxicologists, and forensic engineers allows solving the question of whether drivers are drug/alcohol impaired during driving.

As part of the same protocol, drivers hospitalized after a crash should be subjected to blood and urine sampling for clinical purposes and toxicological screening. Blood and urine must be preserved at -20°C for the next forensic toxicological step. The toxicological tests could highlight if alcohol and drugs—and their metabolite—are present in the blood and in what concentrations. However, we must be careful because, at the current time, specific drug concentration levels cannot reliably be equated with a specific degree of driver impairment. Moreover, as time elapses between a car crash and the medical examination/biological samples collection, the statement and the roadside tests of a law enforcement officer become crucial to help assess whether a driver is impaired. Officers and paramedics should be trained to identify signs and symptoms of drivers impaired by drugs other than alcohol. Hence, a blood test must be able to link drug presence to the observed impairment. Finally, the timing and dynamics of the impacts should always be monitored by a forensic engineer.

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2. Borriello R., Carfora A., Petrella R., Cassandro P. Trends of ethanol and illicit drug use among drivers involved in traffic crashes in Campania (Italy) during 2009-2014. *Proceedings of the 53<sup>rd</sup> International Association of Forensic Toxicologists*; 2015, Florence (Italy) 213.

### Forensic Science, DUI, DUID

## K20 AH-7921 and U-47700 Series Analogs: Spectroscopic Characterization and $\mu$ -Opioid Receptor Pharmacology

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**Learning Overview:** After attending this presentation, attendees will know about the pharmacological differences between the structurally related emerging synthetic opioids, AH-7921 and U-47700.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of publicly deposited spectral information on two series of emerging synthetic opioids. This presentation will also highlight the pharmacological differences of the A-7921 analogs vs. the U-47700 analogs that may have bearing on the relative harm between the otherwise equipotent opioid series.

The published pharmacology for AH-7921 and U-47700 is more than 35 years old and was performed using non-human receptor systems.<sup>1-5</sup> This study synthesized and characterized 19 analogs of the AH-7821 and U-47700 series opioids. The spectra were deposited in publicly available databases for reference purposes and this study assessed their pharmacology using a cloned human  $\mu$ -Opioid Receptor (hMOR) in an *in vitro* stable human cell line. This was to establish the potential for abuse of these analogs and to explore differences in ligand bias in these series.

Analogs within the AH-7921 and U-47700 series were synthesized and characterized by Nuclear Magnetic Resonance (NMR), Gas Chromatography/Mass Spectrometry (GC/MS), Infrared (IR), and Raman (1,064nm). In the U-47700 series, the stereochemically pure *R,R* and *S,S* isomers were synthesized from single isomer intermediates. These spectroscopic data have been deposited in publicly accessible repositories: Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) (GC/MS data & monographs) and “Ethylenediamine synthetic opioids,” Mendeley Data, v1 <http://dx.doi.org/10.17632/tjm8x4m93k.1>.

Full concentration response curves, representing  $G_{\text{oi}}$  suppression of forskolin-stimulated adenylate cyclase, were obtained for morphine, AH-7921, and U-47700 (*R,R* stereochemistry). The  $EC_{50}$  values were 39nM, 27nM, and 9nM, respectively. These values are in agreement with the potencies reported in earlier literature. The (*S,S*) isomer of U-47700 had significantly less potency at this receptor. The literature suggests that these analogs bind to both  $\mu$ - and  $\kappa$ -opioid receptors (OPRM1 & OPRK1) but with differing affinities based on their stereochemistry.<sup>3</sup> In both AH-7921 and U-47700 series, the 3,4-dichlorobenzoyl substitution consistently demonstrated the greatest potency relative to other substitutions on the benzoyl group. Interestingly, U-47700, having the methyl group on the amide removed (Udes), retained good potency ( $EC_{50}$ =3nM), while other Udes analogs did not have a significant naloxone-reversible effect at 1 $\mu$ M.

In the AH-series, the rank order potency was as follows: 3,4-dichloro > 4-chloro > 4-trifluoromethyl > others not showing significant naloxone-reversible effect at 1 $\mu$ M. In the U-series, the rank order potency was as follows: 3,4-dichloro > 4-trifluoromethyl > 4-chloro; 4-methoxy; 4-bromo > others not showing significant naloxone-reversible effect at  $\mu$ M.

Considering that different opioid structural groups exhibit differing profiles of analgesia vs. adverse effects (respiratory depression, hyperalgesia and addictive potential, tolerance, etc.), this study explored the pharmacology of these analogs further. Receptor internalization was assessed for active analogs as a measure of  $\beta$ -arrestin recruitment.  $\beta$ -arrestin recruitment has been associated with adverse effects of opioids. Different opioid ligands display differing abilities to signal via G-proteins vs.  $\beta$ -arrestin recruitment. There is current debate whether biased ligands or ligands with balanced activities would produce the best analgesic/safety profile.<sup>6</sup> Regardless, this data show that AH-7921 leads to only modest levels of internalization similar to morphine, whereas U-47700 leads to high levels of receptor internalization, similar to the opioid peptide DAMGO.

In conclusion, these structurally similar compounds exhibit completely opposite signaling bias. These observations could indicate differences in the safety of these two emerging synthetic opioids that should be explored.

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### Synthetic Opioids, AH-7921, U-47700

## K21 Screening of New Psychoactive Substances (NPS) in Human Plasma Using Magnetic Solid Phase Extraction (m-SPE) by Liquid Chromatography/Quadrupole Time-of-Flight/Mass Spectrometry (LC/qTOF/MS)

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**Learning Overview:** After attending this presentation, attendees will better understand the usefulness of m-SPE for new NPS in human plasma by LC/qTOF/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the usefulness of m-SPE for determining NPS in plasma by LC/qTOF/MS.

The numbers of NPS, including synthetic cannabinoids, phenethylamines, piperazines, and tryptamines, are continuously growing and the identification of these new drugs in biological fluids has been a big challenge in forensic toxicology. To isolate a variety of NPS in biological fluids, Protein Precipitation (PPT), Liquid-Liquid Extraction (LLE), and SPE were commonly used. To develop a new screening method, m-SPE is applied to isolate 41 NPS in this study. In m-SPE, a magnetic sorbent material is dispersed into the sample solution. The sorbent is collected from the sample solution with the application of an external magnetic field.

Forty-one NPS (13 synthetic cannabinoids: AM-1248, STS-135, AM-2233, AKB-48, 5F-AKB-48, APICA, AB-PINACA, QUPIC, 5-Fluoropentyl-3-pyridinoylindole, JWH-307, CB-13, A-836,339, and mepirapim; 12 phenethylamines: PMMA, MMDA-2, 5-APDB, 5-MAPB, 2C-NBOMe, 3-fluoromethamphetamine, 5-IT, DMMA, 25I-NBOH, 5-EAPB, 2C-C, and 2C-P; 5 Tryptamines: 5-MeO-DALT,  $\alpha$ -methyltryptamine, NMT, 5-MeO-EPT, and 4-OH-DET; 4 other substances:  $\alpha$ -PVT, Desoxy-D2PM, Ethylphenidate, and RH-34; 3 aminoindanes: MDAI, 5-IAI, and N-methyl-2-AI; 2 piperazines: NT-45 and 2,3-DCPP; and 1 phencyclidine-type substance: N-ethyl-norketamine) were spiked at 50, 100, 500, and 1,000ng/mL in plasma specimens for the development of this method. The extraction of analytes was performed by m-SPE as sorbent using COOH-functionalized multi-walled carbon nanotubes with magnetic nanoparticles (COOH-mMWCNT). The 125 $\mu$ L of sorbent was dispersed into a 200 $\mu$ L plasma by vortex to form a homogeneous suspension. The plasma was discarded after the sorbent was separated by the external magnet. Then the analytes were desorbed with 1ml elution solvent (8% formic acid in acetone: toluene (3:1, v/v)) by sonication and vortex. After that, the dispersed particles were gathered by the external magnet. The eluate was evaporated by nitrogen gas. The residue was reconstituted with 100 $\mu$ L methanol and analyzed by LC/qTOF/MS (Agilent® 1290 series LC and the Agilent® 6545 QTOF). Chromatographic conditions were as follows: mobile phase A consisted of 0.1% formic acid in water. Mobile phase B consisted of acetonitrile. Separation was achieved in 1 min. Data were acquired in full scan MS mode within the mass range m/z 50–500 at a rate of 250ms/spectrum.

In this study, m-SPE was successfully applied for the extraction of all types of NPS in the human plasma sample. This extraction method is less time-consuming and laborious than conventional cartridge SPE; also, sorbent collection does not require centrifugation. The screening method allowed the detection and identification of 41 targeted compounds by LC/qTOF/MS. Validation was performed for 41 NPS and showed acceptable limits of detection, recovery, matrix effect, and precision. The limits of detection of the 41 analytes were between 0.002 and 0.084 $\mu$ g/mL. An acceptable recovery of synthetic cannabinoids, phenethylamines, tryptamines, other substances, aminoindanes, piperazines, and phencyclidine-type substances were 57–103% (average 86), 80–96% (91), 86–89% (87), 57–98% (84), 39–88% (71), 87–92% (89), and 97%, respectively. Matrix effect ranged from 82–94% (average 88) for synthetic cannabinoids, 79–99% (94) for phenethylamines, 76–102% (92) for other substances, 90–97% (93) for tryptamines, 87–90% (88) for aminoindanes, 92–96% (94) for piperazines, and 82% phencyclidine-type substances. Both intra- and inter-day precision were < 27% (Relative Standard Deviation (RSD)%).

Based on this results, it was concluded that this method is applicable to detect many types of NPS in biological fluids with a short extraction time, low consumption of organic solvent, and easy operation. The detection of metabolites of NPS by this method will be studied in the future.

### Magnetic Solid Phase Extraction, New Psychoactive Substances, Carbon Nanotubes



## K22 Evaluation of Sample Preparation Techniques for the Detection and Quantitation of Benzodiazepines in Human Urine and Whole Blood Using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC-MS/MS)

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**Learning Overview:** After attending the presentation, attendees will be able to use the methods developed, or develop their own singular method, for urine and blood analysis of selected benzodiazepines.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the analysis of selected benzodiazepines using various matrices and sample preparation methods.

**Background/Introduction:** Benzodiazepines are a class of drugs generally prescribed for treatment of anxiety, depression, and insomnia. The three most commonly encountered benzodiazepines, alprazolam, diazepam, and clonazepam, reside in the top 25 most frequently identified drugs based on the United States Drug Enforcement Administration's National Forensic Laboratory Information System 2018 Mid-Year Report and therefore remain of forensic toxicological importance.

**Objective:** The purpose of this research was to evaluate three sample preparation methods, Liquid-Liquid Extraction (LLE), Solid Phase Extraction (SPE), and Supported Liquid Extraction (SLE), for the reliable and accurate identification of six benzodiazepines in human urine and whole blood, including: alprazolam, alpha-hydroxyalprazolam, clonazepam, etizolam, diazepam, and 7-aminoclonazepam. Further, analytical methods were validated using all three of these techniques.

**Method:** A six-point calibration curve and three Quality Control (QC) samples, each in triplicate, were extracted using SLE with ISOLUTE® cartridges, SPE using Clean Screen® XCEL I cartridges, and LLE. Samples were analyzed on a High-Performance Liquid Chromatograph (HPLC) with a 4000 Q-Trap Electrospray Ionization Tandem Mass Spectrometer (ESI/MS/MS) in positive ionization mode. The method was validated in accordance to the proposed AAFS Standards Board (ASB) Standard 036, First Edition 2018, for quantitative analysis by evaluating calibration model, precision, bias, Limit Of Detection (LOD), Limit Of Quantitation (LOQ), carryover, interferences, and ionization suppression and enhancement.

**Results:** With this developed method, analysis time totaled nine minutes. A linear dynamic range of 10–1,000ng/mL was used for all analytes. Recovery of all analytes utilizing the SLE sample preparation method for urine and blood ranged from 56.44–87.73 and 26.13–82.87%, respectively; SPE sample preparation method for urine and blood ranged from 36.95–64.5 and 46.66–79.23%, respectively; and LLE sample preparation method for urine and blood ranged from 44.68–143.9 and 36.17–117.61%, respectively. The LOD utilizing SLE for urine and blood ranged from 0.5–1 and 1–5ng/mL, respectively; SPE for urine was 1ng/mL and blood ranged from 1–2ng/mL; and LLE for both urine and blood ranged from 0.5–1ng/mL.

**Conclusion/Discussion:** The development and validation of these sample preparation and analytical methods demonstrates sensitive, reliable, and reproducible results to identify and quantify six commonly encountered benzodiazepines in human urine and whole blood in rapid time. The use of LLE proved to be efficient; however, sample preparation was laborious and costly. The SLE cartridges used were approximately twice as expensive as SPE, but were the fastest method to prepare samples, whereas SPE consumed a large amount of solvent and took the most amount of time to prepare samples. Given the data discussed above, it is left up to the individual laboratories and analysts to determine which extraction method best suits their needs and current resources.

*This work was supported by the National Institute of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.*

### Benzodiazepines, Sample Preparation, HPLC-MS/MS



## K23 Calculation of Potential Lactate/Lactate Dehydrogenase (LDH) Interference With Alcohol-Dehydrogenase (ADH)-Based Ethanol Assay

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the potential for lactate/LDH interference in an ADH-based ethanol assay, an approach for consideration of the possibility of such interference in a particular case sample based on clinical parameters, and calculation of the magnitude of interference based on estimated, extrapolated, or determined lactate and LDH concentrations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an approach for the evaluation of potential lactate/LDH-based interference in an ADH-based ethanol assay, and for the determination of the magnitude of such interference in specific cases.

Determination of serum alcohol is a common and routine clinical test, most often performed by an enzymatic assay based on the ADH-catalyzed oxidation of ethanol to acetaldehyde with the concomitant reduction of NAD<sup>+</sup>, with the rate of NADH formation being the monitored variable (A340). Interference with an ADH-based alcohol assay by the presence of variable concentrations of lactate and LDH was demonstrated experimentally. Theoretically, the presence of adequate quantities of both lactate and LDH in a sample could result in a competitive, and interfering, reduction of NAD<sup>+</sup>, with the potential for falsely elevated or false positive alcohol results.<sup>1,2</sup> The question of the potential occurrence and magnitude of this phenomena has been the subject of a number of papers. Recently, several well-documented examples of the lactate/LDH interference with an alcohol result have demonstrated the significance such results can have with regard to forensic casework. Interference was found to be a function of variable lactate and LDH combinations, and minimum concentrations required to produce a forensically significant result (e.g., >0.0g/dL) were determined. A scaled-up version of an automated analyzer method, with volumes adjusted for manual analysis in a Ultraviolet/Visible (UV/Vis) spectrophotometer, was utilized with 0–50mM lactate (normal range ~0.5–2.2mM) and 0–10kU/L LDH (normal range ~0.12–0.22kU/L). Each lactate/LDH combination was run in triplicate, with the “Ethanol (EtOH) reading determined from a 6-point ethanol calibration curve. To generate an apparent alcohol result of 0.02g/dL at the maximal lactate concentration (50mM), an LDH of at least 4kU/L was required in the sample, and similarly, at maximal LDH concentration (10kU/L), a lactate concentration of at least 10mM was required. These results are consistent with the uncommon nature of this phenomena, suggesting that significant lactate and LDH levels are required in a sample to cause an interference. Perhaps more relevant, a clinical picture that would be expected to result in a persistently elevated lactate level, combined with significant leakage and presence in the blood of hepatic enzymes (e.g., LDH) would not be common (but can clearly occur as a consequence of disease or trauma).<sup>3</sup> The apparent EtOH readings in the lactate/LDH amended samples was linearly related to lactate concentration at specific LDH levels. Similarly, the slopes of those lines were linearly related to LDH concentration. Using these relationships (expected to be generally applicable, but assay-specific), an equation was derived that relates lactate, LDH, and expected “false-positive” ethanol readings, and describes the potential application of this approach to other assays. Finally, this equation was applied to the analysis of well-documented clinical cases, along with the consideration of relevant clinical parameters and assay results.

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### Lactate, Lactate Dehydrogenase, Alcohol Dehydrogenase





## K24 Fatal Unintentional Cocaine Overdose: The Importance of a Forensic Scene Investigation

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**Learning Overview:** After attending this presentation, attendees will understand the importance of a forensic site inspection in a case of overdose cocaine abuse. Hyperthermia is difficult to find in postmortem, as cocaine overdose deaths are not always immediately discovered, and the body remains exposed to thermal changes for a long time.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about the interpretation of hyperthermia by cocaine-related deaths, which is still difficult and disputed. One of the reasons why literature offers few toxicological data about hyperthermia by cocaine involving death is data lacking about forensic scene investigations.

The correlation between mortality from cocaine and high ambient temperature is widely documented in scientific literature. Cocaine use is correlated with serious toxic effects and death. Subjects who use cocaine on a continuous basis have higher levels of circulating catecholamines. This condition manifests itself clinically as tachycardia, arterial vasoconstriction, enhanced thrombus formation, mydriasis, psychomotor agitation, seizures, coma, and even death. Cocaine modifies dopamine receptors and transporters involved in body temperature control. In fact, the substance determines cutaneous vasoconstriction at the peripheral level, while centrally interfering with the dopaminergic centers at the hypothalamic level, which are responsible for the regulation of body temperature. Hyperthermia is a well-known complication of cocaine use. However, this clinical finding is difficult to find in postmortem as cocaine overdose deaths are not always immediately discovered, and the body remains exposed to thermal changes for a long time. Therefore, the number of deaths due to hyperthermia related to cocaine abuse is quite limited. This study describes a case of cocaine-associated hyperthermia that resulted in death. A 33-year-old man was a known cocaine user. The decedent was found dead in his house by his live-in wife. The woman said the man had been using cocaine mainly by inhalation for about five years, had long been depressed, and did not leave home anymore. The wife also reported that around 8:00 p.m. she was outside with her children, and when she came back home, she discovered her husband lying on the floor of the house.

Rectal postmortem temperature (11:26 p.m.) was 41.7°C, compared to an ambient temperature of 30°C (11:30 p.m.). Hypostasis was abundant and disappeared on pressure; rigor mortis was in progression from small to large articulations. The autopsy examination showed only polyvisceral congestion; histological examinations documented myocardial fibrosis related to an initial dilated cardiomyopathy. No macroscopic abnormalities were observed in the other organs. During the forensic site inspection, three small packages containing white powder were found, later analyzed, and determined to be cocaine and lidocaine.

General drug screening conducted on a blood sample provided positive results only for residual cocaine (5.68ng/mL). Cocaine, Benzoylecgonine (BE), Ecgonine Methyl Ester (EME), and cocaethylene were measured in cardiac blood by gas chromatography and mass spectrometry (EME 1.34µg/mL, BE 4.78µg/mL, cocaethylene 0.021µg/mL), demonstrating high blood concentrations of the metabolites (6.12µg/ml), suggestive of a massive administration four to five hours before death. Considering the temperature measured during the site inspection about four hours after death, (already 41.7°C), it is hypothesized that at the time of death the victim had reached a hyperthermal peak of approximately 43–44°C.

This is a rare case in which it was possible, through the site inspection where the death body was found and the simultaneous measurement of the temperature, to identify the cause of death as hyperthermia resulting from massive cocaine intake, with no evidence of macroscopic and microscopic alterations that could justify death.

### Hyperthermia, Site Inspection, Cocaine



## K25 Presumptive Identification of Nitrite by Griess Reagent Test Strips—Applications in Suicide Investigations

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**Learning Overview:** After attending this presentation, attendees will understand the utility of a simple, commercially available color test to triage potential sodium nitrite poisoning cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the use of a supplemental presumptive screening tool for an analyte that is not conventionally included in forensic toxicology panels. This knowledge may prevent expensive, undirected private laboratory testing while confirmatory methods are developed or laboratories with validated ion monitoring procedures are identified.

Nitrite poisonings have been documented for decades, with incidents including fertilizer contamination of well water, ingestion of improperly cured meats, and infant exposure to vegetables high in nitrites and nitrates. More recently, sodium nitrite and sodium azide have been advocated by online suicide forums as an effective means of fatal overdose. Three cases submitted to the University of Florida (UF) Forensic Toxicology Laboratory initially indicated suspected sodium nitrite poisoning. The fourth case underwent exhaustive toxicological testing with inconclusive test results before scene evidence implicated intentional sodium nitrite ingestion.

Spectrophotometry, ion chromatography, and Gas Chromatography/Mass Spectrometry (GC/MS) methods exist for the quantitation of nitrite and nitrate ions and are commercially available for postmortem livestock samples. However, such testing involves specialized detection equipment and/or preparation methods that are not part of standard human toxicological testing schemes, including those of the UF laboratory and its reference laboratory. Until validated methods are developed, it was hypothesized that a common Griess reagent color test (MQuant™ Nitrite Test Strips) could be used as a presumptive triage method in cases where ingestion of sodium nitrite is suspected.

Initial testing was performed on four suspected nitrite ingestion cases using available clear fluid samples. The unconfirmed presumptive test results are presented below:

Case	Sample Type	Presumptive Test Result
Negative Control	Urine	None Detected
20mg/L Standard	Aqueous (Water)	Positive
5mg/L Control	Urine	Positive
Case 1	Urine	None Detected
	Vitreous Humor	Positive
	Serum	None Detected
	Stomach Contents	Positive
	White Crystals from Water Bottle	Positive
Case 2	Urine	Positive
	Vitreous Humor	Positive
	Stomach Contents	Positive
Case 3	Urine	Positive
	Vitreous Humor	Positive
Case 4	Vitreous Humor	Positive
	Serum	None Detected
	Stomach Contents	Positive

The test strips provide a color scale for the semi-quantitative measurement of nitrite concentrations between 2 to 80mg/L, a range higher than typical urine concentrations in healthy individuals. While the test strips used in this study are intended for the monitoring of nitrite in water, the same Griess reagent color test is routinely performed in urinalysis panels indicated for the detection of urinary tract infections and endocrine disorders.

Due to the ubiquitous nature of nitrogen oxides, their association with common diseases, such as diabetes, and potential interferences (chromate, permanganate, other oxidants), test results must be interpreted with caution. Of 27 randomly selected postmortem cases of varying sample quality and exogenous content, none produced positive test results in serum, urine, vitreous humor, or stomach content samples. Therefore, in conjunction with physical indicators such as brown discoloration of the blood and cyanosis from peri-mortem methemoglobinemia (MetHb), medical history, and/or scene evidence, nitrite screening test kits can provide toxicologists with insight on suspected nitrite poisoning cases.

### Nitrite, Suicide, Presumptive Testing



## K26 The Detection and Quantification of Tianeptine in Postmortem Blood and Urine

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**Learning Overview:** After attending this presentation, attendees will be familiar with the toxic and lethal effects of tianeptine and a method for its toxicological analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by relaying information about the toxicity of tianeptine, an atypical tricyclic drug.

**Proposition Statement:** The presented postmortem concentrations of tianeptine are consistent with a fatal overdose.

**Background/Introduction:** Tianeptine is an atypical tricyclic drug approved for use in Europe, Asia, and Latin America. In the United States, tianeptine is not approved by the United States Food and Drug Administration (FDA), but is sold on websites as an unscheduled pharmaceutical agent, a nootropic or smart drug/cognitive enhancer. Tianeptine use in the United States has increased in the last several years. The National Poison Data System received a total of 11 tianeptine exposure calls between 2000 and 2013. The total number of tianeptine exposure calls increased from 5 in 2014 to 38 in 2015, 83 in 2016, and 81 in 2017. The dual activation of the mu and delta opioid receptors is believed to be responsible for causing many of the known acute and chronic effects of tianeptine, including its antidepressant and anxiety actions. The major metabolic pathway is  $\beta$ -oxidation and the principal metabolites are propanoic acid (inactive) and pentanoic acid (active) metabolites. Less than 3% of the dose is excreted unchanged in urine. There are limited published case reports of tianeptine fatal overdoses with blood concentrations ( $n=5$ ) ranging from 2–18mg/L and free tianeptine in urine ( $n=2$ ) at 2.0 and 3.2mg/L.<sup>1-4</sup>

**Case:** Presented are the femoral blood and urine results from a 31-year-old White male found at home by his girlfriend approximately four hours after he was last seen. Needles, syringes, and a glass pipe were also found at the scene. Initial screening of the blood and urine samples revealed amphetamine, methamphetamine, and tianeptine.

**Method:** A High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS) method was used for the detection and quantitation of tianeptine in blood and urine. A seven-point matched matrix calibration curve ranging from 0.001–0.100mg/L, blood and urine quality control specimens (0.001, 0.003, 0.030, and 0.075mg/L), and the samples were analyzed. Method was assessed for bias, precision, carryover, matrix effects, and dilution integrity. The linear regression coefficients of determination ( $r^2$ ) for calibration curves were 0.9996 or greater. Controls were assessed over three days in triplicate. Accuracy/bias of the quality control specimens was determined to be within  $\pm 20\%$  of the target concentrations for both blood and urine. The precision did not exceed a coefficient of variation of 15%. Validation criteria for carryover, specificity, matrix effects, and dilution integrity were also acceptable. Blood samples were deprotonated with methanol prior to extraction. To prepare the samples, 0.200mg/L protriptyline Internal Standard (ISTD) was added to 200 $\mu$ L aliquots of the calibrators, controls, and samples followed by the addition of 200 $\mu$ L of 50:50 acetonitrile: water. Samples were briefly mixed and transferred to Clean Screen FAST<sup>®</sup>SPE columns and rapidly eluted into auto-sampler vials. Analysis was performed using a Waters<sup>®</sup> ACQUITY<sup>®</sup> Xevo Tandem Quadrupole Detection (TQD) HPLC/MS/MS with ESI ionization in positive ion mode.

**Results:** The determined blood and urine concentrations were 11mg/L and 0.66mg/L, respectively.

**Conclusion:** The blood and urine tianeptine concentrations determined by the presented HPLC/MS/MS method were consistent with the limited published tianeptine fatal overdose concentrations. The blood concentration of 11mg/L was within the range of published blood tianeptine concentrations. The urine concentration of 0.66mg/L was lower than that of the two published urine postmortem tianeptine concentrations.

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### Tianeptine, Postmortem, HPLC-MS/MS



## K27 Attribution Signatures for the Sourcing of Dokha and Dokha-Infused Tobacco Products

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**Learning Overview:** The goal of this presentation is to educate the viewers on dokha, a new tobacco product and how to attribute that product back to its vendor.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing the feasibility of using attribution and discriminant function analysis models and their impact on drug analysis. This presentation will also provide insight into an emerging drug and its elemental content.

Dokha is a tobacco product that is primarily consumed in the Middle East.<sup>1</sup> It is comprised of dried tobacco leaves, spices, flowers, and other herbs.<sup>1</sup> The tobacco for dokha is manufactured by drying fine tobacco leaves in the desert region.<sup>2</sup> This processing method tends to lead to high levels of nicotine and carbon monoxide in dokha and associated products.<sup>2</sup> Dokha is typically smoked through a pipe called a midwakh, which is capable of holding approximately 0.5 grams in a small bowl.<sup>1</sup>

Dokha belongs to a class of alternative tobacco products that includes items such as shisha, hookah, and vape pens.<sup>3</sup> There is currently a rise in alternative tobacco products as there is a reported decline in cigarette usage.<sup>3</sup> However, some of these products, like dokha, could be more harmful than cigarettes. Past research has shown that dokha tends to have significantly higher levels of nicotine when compared to certain brands of cigarettes.<sup>2</sup> There has also been research that shows dokha having potential linkages to seizure activity.<sup>4</sup> This bodes as a concern as dokha has recently made its way to the United States. In fact, certain brands can be found at various local smoke shops. Dokha itself is regulated by the Food and Drug Administration (FDA) as a tobacco product and regarding package labeling; however, it is not a scheduled drug by the Drug Enforcement Agency (DEA).

The attribution of dokha and dokha-related products could be useful in cases of overdose. The hypothesis for this study is that utilizing the elemental content, a model can be made that will allow for sourcing back to the associated vendors. It is well known that certain elements, such as chromium, lead, and cobalt, are of great concern as carcinogens.<sup>1</sup> This study also helps to provide insight into some of those elements and their concentrations in these products. A total of four vendors were evaluated for this analysis and one vendor was also evaluated for the dokha-infused hookah product that they sell. This additional dokha-infused hookah was chosen for evaluation because hookah is a particularly popular recreational drug of consumption in the United States. To retain continuity between the brands, only the unflavored type of each brand was purchased from the vendors' respective websites. Each dokha sample was digested in triplicate in an overnight digestion procedure consisting of 7N nitric acid. The Inductively Coupled Plasma/Mass Spectrometer (ICP/MS) was the instrument used to obtain the inorganic elemental data. A discriminant function analysis plot was used to establish a model for separation between each separate brand of dokha based on their respective elemental contents.

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### Attribution, Dokha, ICP/MS



## K28 Assessment of *In Vitro* Methemoglobinemia Formation in Infant Samples

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**Learning Overview:** After attending this presentation, attendees will have learned the caveats of postmortem methemoglobin interpretation in adult and fetal postmortem blood and the mechanisms to circumvent these limitations for proper interpretation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing a large dataset from a national reference laboratory for methemoglobin trends in adults and infants with guidance on interpretation of results.

**Hypothesis:** Interpretation of methemoglobin percentage in postmortem samples, specifically infant samples, is complicated by *in vitro* formation and susceptibility of fetal hemoglobin to oxidation in the absence of an exogenous oxidant.

**Content:** The review of historic methemoglobin results in postmortem samples collected from infants illustrated a wide distribution of results and difficulty with interpretation. Redevelopment of an existing forensic method to allow its use in clinical methemoglobinemia determination resulted in the identification of extreme elevations of methemoglobin in postmortem samples previously undetected. Suspected nitrate/nitrite ingestion was indicated for many of the elevated adult methemoglobin results and nitrate/nitrite levels via an enzymatic method were assessed in several specimens to confirm. Elevated methemoglobin results in infant samples did not routinely correlate with case histories and were proposed to be falsely increased due to a spectral interference. However, residual fetal blood samples collected from umbilical cords of otherwise healthy infants had minimal methemoglobin present, decreasing the likelihood of a broad interference due to fetal hemoglobin contributing to the high percentage of elevations observed. Oxidation studies were conducted with stored blood from adult and fetal origin, with and without an oxidizing agent present, to illustrate the relative susceptibility of fetal hemoglobin to oxidize post-collection.

**Results:** Historic data were analyzed to demonstrate the distribution of results before and after redevelopment of an existing methemoglobin method using spectrophotometry and ranged between <1% to >80%. Elevations in methemoglobin percentages in adult postmortem samples consistent with case presentation were confirmed for the presence of nitrate/nitrite using an enzymatic method. Fetal and adult samples were further assessed to evaluate the formation of methemoglobin during storage in the absence and presence of oxidizing agents with studies currently in progress.

**Conclusion:** Interpretation of postmortem methemoglobin results requires caution, specifically in infant samples. However, follow-up testing based on case presentation provides the necessary context to interpretation of the results from postmortem blood.

**Methemoglobin, Pediatric Toxicology, Nitrate/Nitrite**



## K29 Drug Trends in Korea and the Detection of Synthetic Cannabinoids in the Hair of Drug Abusers

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**Learning Overview:** After attending this presentation, attendees will understand the drug trends in Korea and the detection of synthetic cannabinoids in the hair of drug abusers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing drug trends in Korea and data from the detection of synthetic cannabinoids in the hair of drug abusers in three recent years (2016–2018).

**Introduction:** Before the 1980s, opium and methadone were widely used in Korea. Since the 1970s, marijuana has become a public health concern, especially for entertainers. Methamphetamine has also spread remarkably in the domestic market and become the most frequently abused drug in Korea from the 1980s to the present. The first of the New Psychoactive Substances (NPS) appeared in Korea in 2009 and has increased rapidly. This study investigated the drug trends in Korea and the results from the detection of synthetic cannabinoids in the hair of drug abusers for three recent years (2016–2018).

**Methods:** The drug trends in Korea were referenced from the Drug Crime Report 2018 published by the Supreme Public Prosecutor's Office. The numbers of arrested drug criminals, arrests by drug crime type, arrests by the criminal's age, and arrested foreign drug criminals and their nationalities were investigated. The method used to analyze synthetic cannabinoids in hair samples was conducted according to the standard operation procedure of the National Forensic Service (NFS). In brief, the drugs in hair samples were extracted in ethanol and analyzed by liquid chromatography-tandem mass spectrometry. In total, 59 synthetic cannabinoids (18 synthetic cannabinoids and 41 of their metabolites) were screened.

**Results:** The number of arrested drug criminals in Korea has exceeded 10,000 since 2015 and was 12,613 in 2018. The number-one type of drug crime was "use" (making up 49% of the total), followed by "trafficking" (22%) and "possession" (8.6%). In contrast, "manufacture" was the lowest drug crime at 0.1% of the total. Criminals in their 30s and 40s made up 50% of the total. The number of foreign criminals had increased by 220%, from 295 in 2011 to 948 in 2018. Among them, nationals of China were the highest in number (making up 39% of the total), followed by nationals from Thailand (32%), the United States (9%), Taiwan (5%), Vietnam (3%), and Uzbekistan (2%). In 2018, methamphetamine was the drug that was confiscated the most (187,947g), followed by cannabis (89,145g) and cocaine (88,321g). Meanwhile, according to the NFS, the number of seized materials containing NPS at NFS has been increasing rapidly until 2014, and then decreasing gradually. In the three years (2016–2018), the most frequently detected synthetic cannabinoid in the hair of drug abusers was AB-CHMINACA, with a total of 32 cases detected. From 2016 to 2018, the number of cases with synthetic cannabinoids detected in the hair samples was 25, 24, and 4, respectively, indicating that it had decreased significantly in 2018.

**Conclusion:** Currently, although the most abused drug in Korea is still methamphetamine, the types of drugs being abused are becoming more diverse, especially with the recent emergence of NPS. The amount of cocaine confiscated in 2018 was high, which is considered unusual in Korea, where there are few cases of cocaine abuse. We need to look at the trends for the next few years. The number of synthetic cannabinoids detected in hair samples in 2018 was lower than that of previous years. It seems that newly abused NPS were not detected in the existing analysis. Because the structure of NPS changes so fast, it is difficult to reflect new changes with the existing screening methods established in the laboratory. Therefore, there is an urgent need to establish a method for detecting newly abused NPS (e.g., 5F-ADB, FUB-AMD, etc.) in biological specimens.

### Drug Trend, New Psychoactive Substances, Synthetic Cannabinoids



## K30 Electrochemical Detection of Fentanyl Using Screen-Printed Carbon Electrodes With Confirmatory Analysis of Fentanyl and Its Analogs in Oral Fluid Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Overview:** After attending this presentation, attendees will be able to explain the theory behind the use of electrochemical techniques such as Square-Wave Voltammetry (SWV) for the analysis of fentanyl and illicit substances in forensic science and will understand the importance of these techniques as screening tools.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing new methods of screening illicit substances both at the crime scene and within the lab that are fast, reliable, and provide both qualitative and quasi-quantitative data. This work may aid in speeding up and improving the seized drug workflow on cases, helping to reduce backlog and increase the speed of investigations.

Due to the growing number of cases involving fentanyl and fentanyl analogs, opioid abuse poses a significant threat to the United States. Opioid-related overdose deaths have increased over the past several years leading to a public health emergency declared by the Department of Health and Human Services. New Psychoactive Substances (NPS) have compounded the issue due to having similar or increased potency. NPS are synthetic analogs to known controlled substances designed by making modifications to the core chemical structure in most cases, and include fentanyl-analogs and synthetic cannabinoids, among others. Electrochemistry can provide a rapid, sensitive, and selective screening technique to overcome the limitations faced by other methods in the field. Electrochemistry offers a versatile platform that is sensitive, portable, and low cost, which can be modified to suit a variety of needs and detection requirements.

Utilizing Screen-Printed Carbon Electrodes (SPCEs), a fast, simple, and sensitive approach toward the detection, identification, and quasi-quantitation of fentanyl, was achieved both in an electrochemical cell and as a drop on the electrode surface. Electrooxidation of fentanyl at the electrode was demonstrated using SWV between -0.5V and +1.6V with 100mM Tris-HCl buffer at pH 8.5 as supporting electrolyte. Parameter optimization was conducted. Voltammograms demonstrated the presence of two oxidation peaks at 750mV (peak I) and 880mV (peak II) versus pseudo Ag/AgCl. Fentanyl oxidation was observed at concentrations of ~76ng/mL in cell and ~300ng/mL in a 100µL drop.

Reproducibility between electrodes, assessed as the average Relative Standard Deviation (RSD), for peak I and peak II in cell was 12% and 18%, respectively. RSD in drop was 13% and 15% for peaks I and II. Accuracy of the detection method was determined in cell by analyzing single-blind samples prepared in lab and demonstrated better accuracy in lower concentrations of fentanyl versus higher concentrations. The assessment of the effects of interfering compounds, including cocaine, methamphetamine, quinine, and acetaminophen, was performed. Analysis of various ratios of fentanyl to interferent were analyzed and demonstrated the ability of the method to detect fentanyl while present in these mixtures. The mechanism of the electrooxidation of fentanyl in the system is proposed herein.

A confirmatory Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for the analysis of fentanyl and fentanyl analogs in oral fluid using dynamic Multiple Reaction Monitoring (dMRM) was developed and validated. A total of 13 fentanyl/fentanyl analogs with 7 internal standards were analyzed. The limit of detection of the majority of drugs was determined to be 0.01ng/mL with the limit of quantitation at the lowest calibrator of 0.1ng/mL with coefficients of determination between 0.9992 and 0.9999. Solid Phase Extraction (SPE) was used in the assessment of bias, precision, matrix effects, recovery, and process efficiency, which were deemed acceptable based on validation guidelines. Selectivity was demonstrated through the analysis of 12 additional illicit substances commonly encountered in the forensic laboratory. Processed sample stability and freeze/thaw stability were also assessed.

### Electrochemistry, Fentanyl, LC/MS/MS



## K31 Frequency of Gabapentin in Postmortem Cases Screened by Enzyme Linked Immunosorbent Assay (ELISA)

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**Learning Overview:** The goal of this presentation is to summarize the prevalence of gabapentin in routine postmortem case work using ELISA screening and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) confirmation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing frequency, concentrations, and drug combination information of gabapentin in postmortem cases. This data will provide laboratories information to help make decisions as to whether or not they should include gabapentin as part of their own testing regimen.

Prior to June 2019, a large percentage of this study's laboratory's postmortem testing, which includes ELISA and high-performance Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF-MS) screens, did not routinely look for the presence of gabapentin. It was only tested for if requested by a client for direct analysis, added onto routine testing because of case history provided by a client but not ordered up front, or part of a certain testing panel that included screening by Gas Chromatography/Mass Spectrometry (GC/MS). Increased demand and prevalence of gabapentin dictated that it was necessary to include it in the scope of analysis for other testing panels. An ELISA kit became available to test for gabapentin and was incorporated into the other postmortem testing panels where the scope of analysis is more than simply drugs of abuse. The reporting limit for the screening analysis by ELISA has been established at 5.0mcg/mL and 1.0mcg/mL for confirmation analysis by high-performance LC/MS/MS, respectively.

After six weeks of collecting data, approximately 11% (681/6,224) of blood samples from cases that would not have previously been screened for gabapentin using the laboratory's previous testing platform were presumptively positive by ELISA. The confirmation rate was 82% (559/681). Observed concentrations of gabapentin during this time period ranged from (1.1-610)mcg/mL, with a mean of 17.9mcg/mL and median of 10.0mcg/mL. Decomposition is suspected to be an interferent with this ELISA kit.

Analysis of positive blood gabapentin case data near the start of the new testing platform revealed that the most frequently seen drug in combination with gabapentin was fentanyl, followed in order by ethanol, cannabinoids, oxycodone, clonazepam and 7-amino clonazepam, hydrocodone, cocaine and benzoylecgonine, amphetamine/methamphetamine, alprazolam, diphenhydramine, and heroin (defined as a case in which 6-monoacetylmorphine was detected in any matrix). Data collected over a seven-month period beginning June 2019 through January 2020, including concentration ranges of gabapentin and frequency of co-administration with other drugs, will be presented.

### Gabapentin, ELISA, Postmortem





## K32 Comparison of Data Acquisition Methods for High Resolution Mass Spectrometry (HRMS) Drug Screening

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**Learning Overview:** After attending this presentation, attendees will understand how HRMS data acquisition can be used to improve strategies for drug detection.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the relative merits of quadrupole Time-Of-Flight-Mass Spectrometer (qTOF-MS) data acquisition approaches and the challenges associated with the technological shift from traditional immunoassay to HRMS-based drug screening.

According to the most recent census of publicly funded laboratories in the United States, forensic toxicology is now the most outsourced discipline in forensic science. The increased need for outsourcing can be attributed in large part to the proliferation of New Psychoactive Substances (NPS) and the expanded scope of drug testing that is required in antemortem and postmortem toxicology investigations. Over several decades, drug users have shifted from classical drugs of abuse, such as cocaine and marijuana, to new emerging synthetic drugs, including designer benzodiazepines, synthetic cannabinoids, cathinones, fentanyl analogs, and novel opioids. Traditional immunoassay-based screening techniques cannot keep pace with these developments due to the sheer number of drugs, need for adaptation, and limitations in terms of their cross-reactivity.

In order to combat this challenge, some laboratories have transitioned to MS-based screening. HRMS using qTOF-MS is of particular interest because of its sensitivity, specificity, and potential for retrospective data analysis. HRMS allows laboratories to keep abreast of the transitory drug trends and can be more readily adapted to the changing landscape of drug use than traditional immunoassay-based screening. This study focuses on the optimization of data acquisition techniques using Liquid Chromatography (LC) qTOF-MS. Three different acquisition modes were evaluated for this purpose, including TOF MS, All Ions MS, and Auto MS/MS. TOF-MS functions as a traditional time of flight instrument using a precursor ion for detection. All Ions mode MS simultaneously analyzes all precursor ions in addition to fragmenting each precursor at set collision energies, thus providing an additional layer of identification with fragment confirmation. In contrast, Auto MS/MS mode isolates one precursor ion at a time and also fragments each precursor at set collision energies. In addition, the Auto MS/MS mode utilizes an abundance threshold to dictate which precursor ions are fragmented. Using the All Ions approach, all ions that travel through the instrument are fragmented, regardless of abundance. As a consequence, there are relative merits associated with each acquisition mode. The goal of this study was to perform a side-by-side comparison of these techniques using analytes of interest in forensic toxicology. Using certified reference materials, common drugs of abuse and NPS were analyzed using optimized data acquisition methods with an Agilent® 6530 Accurate Mass qTOF-MS. A comparison was conducted for each drug's mass error, target score, and signal-to-noise ratio using each approach. The target score is a weighted value determining how well the mass, isotopic pattern, and retention time of the suspect compound coincide with the target compound.

It was hypothesized that the All Ions data acquisition mode would prove the most robust screening tool since fragmentation occurs for all precursor ions, regardless of abundance. All Ions data acquisition collects and fragments all precursor ions increasing the probability of detecting novel drugs retrospectively as a delay in identification is expected because clandestine production rate surpasses current laboratory identification capabilities. This untargeted approach to screening is highly beneficial in forensic toxicology, given the transient nature of many drug trends. Data-dependent and data-independent acquisition are explored, and the relative merits of these approaches are discussed.

### HRMS, NPS, QTOF-MS



## K33 Analysis of Cannabinoids in Vitreous Fluid

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**Learning Overview:** The goal of this presentation is to present a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method development and validation study for analyzing vitreous fluid for Tetrahydrocannabinol (THC), 11-Nor-9-Carboxy- $\Delta^9$ -Tetrahydrocannabinol (THCCOOH), 11-Hydroxy- $\Delta^9$ -Tetrahydrocannabinol (11-OH-THC), and Cannabidiol (CBD). Casework examples comparing blood and vitreous cannabinoids will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the value of vitreous fluid as a toxicologically important matrix when other matrices are not available.

Cannabis is currently the most widely abused illicit drug in the world.<sup>1</sup> In the United States, recreational cannabis use is legal in 11 states and medical cannabis use is legal in 33 states. As medical and recreational uses of this substance become increasingly legal, there is a need for reliable analytical methods that can detect and analyze cannabinoids in death cases where there is a question relating to the cause and manner of death when typical matrices are not available. Vitreous humor is a gelatinous fluid located in the eyeball and is regularly drawn during autopsy and used as a matrix to test for drugs and alcohol by a postmortem toxicologist. This study utilized bovine vitreous fluid as a blank matrix for method development and validation.

THC is the primary psychoactive component in cannabis and produces the euphoric, relaxing feelings that are associated with the drug. 11-OH-THC is the primary active metabolite of THC and THCCOOH is the secondary and inactive metabolite of THC. CBD is a non-psychoactive component in cannabis that has emerged as a potential remedy for pain, anxiety, and other common health issues. Hemp-based CBD products with less than 0.3% THC content are federally legal in the United States. In this study, a method was developed and validated to quantitate THC, CBD, 11-OH-THC, and THCCOOH in bovine vitreous humor. Samples were prepared by a liquid-liquid extraction using 1 milliliter of vitreous fluid, acidified water, and organic solvents. Separations were conducted using a Phenomenex® Kinetex® C18 (2.1x100 mm, 2.6 $\mu$ m particle size) with VANQUISH™ High Pressure Liquid Chromatography System. Gradient elution was performed with 0.1% formic acid in water and acetonitrile. Identification and quantitation were performed with a TSQ Endura™ Triple quadrupole Mass Spectrometer (QqQ MS) operating in selective reaction monitoring mode. This LC/MS/MS technique combines low complexity sample preparation with the selectivity and sensitivity of LC/MS/MS.

This method was cross validated in whole blood and plasma matrices and used in routine, postmortem toxicology casework. Samples were screened using and Enzyme-Linked Immuno-Sorbent Assay (ELISA) and quantitatively confirmed using the developed LC/MS/MS cannabinoid assay. Limits Of Quantitation (LOQ) are typically 0.5ng/mL with an analytical measurement range reaching 250ng/mL. Casework samples testing positive by ELISA showed the presence of cannabinoid compounds. Blood samples testing positive for cannabinoids showed a positive correlation for trace levels of THCCOOH in vitreous.

This method allows for detection of CBD that can clarify the reported use of federally legal food supplements and/or products used for their reported medicinal benefit. Driving under the influence of marijuana is a major concern in society and postmortem cases involving motor vehicle fatalities need to have forensically valid testing for interpretation of marijuana usage. Furthermore, it is important to differentiate detection of CBD and metabolites versus THC and metabolites in these cases.

### Reference(s):

<sup>1</sup>. United Nations Office on Drugs and Crime, *World Drug Report 2016* (United Nations publication, Sales No. E.16.XI.7).

### LC/MS/MS, Cannabinoids, Vitreous



## K34 Chiral Separation of Methylphenidate, Ethylphenidate, and Ritalinic Acid in Blood

*Christina Smith, BS\*, Red Oak, TX 75154; Madeleine J. Swortwood, PhD, Sam Houston State University, Huntsville, TX 77341*

**Learning Overview:** After attending this presentation, attendees will understand chiral separation of cognitive stimulants and how to effectively isolate and extract them from biological samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the attendees with a unique approach to extraction of cognitive stimulants from blood. With this, a novel approach to chiral separation will be presented to the forensic community.

There has been a recent trend of abusing cognitive stimulants. Drugs such as Methylphenidate (MPH) are commonly prescribed for Attention-Deficit/Hyperactivity Disorder. MPH exists as two enantiomers, though the Dextro (D) configuration is more potent. Analytically, separation of the isomers is essential. However, chiral analysis poses challenges to researchers. Currently, there are no methods available to detect multiple chiral stimulants, and their metabolites in a single analysis. While Ritalinic Acid (RA) is the primary metabolite, Ethylphenidate (EPH) is also produced when alcohol is co-administered. Due to limited assays, this study sought to develop a method that separates the enantiomers of MPH and its metabolites following isolation and extraction from blood samples. Methods such as this are critical to understanding the pharmacokinetics of such cognitive stimulants.

Blood (0.25mL) was fortified with deuterated internal standards (0.025mL). The samples were diluted with phosphate buffer (pH 6) and allowed to stand for five minutes before centrifugation. Following conditioning with methanol and phosphate buffer, samples were loaded onto DAU clean screen extract Solid-Phase Extraction (SPE) cartridges. Cartridges were washed with 0.1M acetic acid and methanol. Analytes were eluted with 2% ammonium hydroxide in methanol, dried, and reconstituted in mobile phase. An Agilent® Technologies 1290 Infinity Liquid Chromatograph coupled to an Agilent® 6470 Triple Quadrupole spectrometer was used for detection of the analytes. Chiral separation was achieved using an Agilent® Poroshell Chiral-V column (2.1 x 100mm, 2.7u) with a flow rate of 0.6mL/min. Mobile phase consisted of deionized water (A) and trifluoroacetic acid (0.0125%, v/v) and ammonium acetate (0.025%, w/v) in methanol (B) at a ratio of 2:98 (A:B) with an isocratic elution. The run time was four minutes.

When developing this method, various parameters were assessed in order to optimize chiral separation. Different gradient elution systems with different buffers and mobile phase composition were investigated in hopes of achieving full baseline separation of the enantiomers. With the final mobile phase and column selections (discussed above), full baseline resolution between the D- and L-MPH enantiomers and the D- and L-EPH enantiomers was achieved. In the same injection, this study was also able to detect and separate RA. Various sample preparation techniques such as protein precipitation, liquid-liquid, supported liquid, and other SPE chemistries were investigated. Optimal isolation and extraction of the analytes in blood was achieved in the extraction discussed above. Recoveries were >90% and matrix effects were <±22% with the exception of RA (80%). All matrix effects and extraction recoveries were compensated by matched deuterated internal standards.

This is the first known method that chromatographically separates the enantiomers of MPH and EPH, in addition to RA. This method also utilized simple SPE to isolate the drug and metabolites from a single sample of blood (0.25mL).

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### Chiral Separation, Methylphenidate, Blood

## K35 Scientific and Careful Setting Up of a Lethal Oleander Leaves Infusion

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**Learning Overview:** After attending this presentation, attendees will know more about determining suicidal death by poisoning through the autonomous preparation and drinking of a *Nerium oleander* leaves infusion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a complete evaluation of the forensic aspects of *Nerium oleander* self-poisoning, analyzing scene investigation, external body examination, autopsy findings, and toxicological and histological analysis.

Suicidal oleander poisoning is uncommon, although some cases are described in the literature.<sup>1</sup>

The uniqueness of this case lies specifically in what the scene investigation findings suggested, namely the scientificity of the act implemented by the man, a laboratory technician, who arranged everything in great detail and in a manner that shows the fully lucid and conscious intention of killing himself. The additional unique element, further supporting full awareness and thorough planning of the act, was the presence on the scene of warnings for “Poison,” showing concern for people approaching the scene after his death. Per research, this is the first report of suicide using an infusion of *Nerium oleander* leaves and the first time toxicological analyses have investigated the ratio of oleandrin concentrations in the vitreous humor versus the cardiac/peripheral blood and other biological samples.

*Nerium oleander* is an indoor and ornamental plant of an evergreen shrub, widespread in Mediterranean countries and one of the most poisonous plants known to humans. All parts are toxic. The effect is primarily due to the cardiac glycosides (oleandrin, nerin, digitoxigenin, and olinerin). Oleandrin is the principal toxin. Clinical manifestations of poisoning appear a few hours after intake and include gastrointestinal, central nervous system, and cardiovascular effects, characterized by nausea, vomiting, salivation, colic, diarrhea, ataxia, drowsiness, muscular tremor, ventricular tachycardia, dysrhythmia, and heart blockage. *Nerium oleander* poisoning can be accidental, homicidal, or suicidal.<sup>2</sup>

Reported here is the case of a suicidal poisoning of a 71-year-old man, which occurred in Castellana Grotte (BA) in March 2019. The scene investigation (the man's house) revealed the presence of a steel pan, with its cover on, sealed with packing tape.

A small piece of white Scotch<sup>®</sup> tape was on the cover, with a note in pen on it: *Poison. Wash pan and funnel carefully or throw everything away.* Inside the pan, elongated dark green leaves, small trunks, and a plastic funnel were found. A smaller pan and a bottle were also present on the scene, both containing a golden yellow fluid. A piece of white Scotch<sup>®</sup> tape was also present on the bottle, with the following note: *Poison.* An empty glass was also located at the scene.

The external examination performed on the man's body revealed blood percolation from respiratory orifices. No other significant findings were encountered.

The autopsy findings showed multi-organ congestion, as is common in cases of oleander poisoning.<sup>3</sup> Moderate congestion was present in the kidneys, liver, brain, and lungs. The lungs also showed some indications of hemorrhagic suffusion. Stomach content showed the presence of a brown liquid (0.5cc). The bowel was resected with absence of the distal portion of the small intestine and the right part of the large one, as the man had been previously operated for cecum carcinoma.

The histological examination of the tissues corroborated edema of the kidneys, liver, brain, and lungs with some indications of hemorrhagic infiltration in the latter, as macroscopically observed. No other abnormalities were revealed.

Toxicological investigations performed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) on the samples collected during the autopsy (blood, urine, gastric content, vitreous humor, liver) confirmed the presence of toxic levels of oleandrin in all specimens.<sup>4</sup> In detail, the results of oleandrin quantification were: blood 37.5ng/ml; vitreous humor 12.6ng/ml; urine 83.8ng/ml; liver 205ng/mg; and gastric content 31.2µg/ml. Oleandrin was also present into the yellow liquid found at the scene, in a concentration of 38.5µg/ml. Hence, the diagnosis of self-poisoning through the ingestion of an infusion of oleander leaves.

This case is a good illustration of the overall set of forensic aspects relating to self poisoning by means of an infusion of the oleander leaves, with the added detail of the meticulous planning of the suicide.

### Reference(s):

1. Azzalini E., Bernini M., Vezzoli S., Antonietti A., Verzeletti A. A fatal case of self-poisoning through the ingestion of oleander leaves. *J Forensic Leg Med.* 2019 Jul; 65:133-136.
2. Julia Radenkova-Saeva, P. Atanasov. Cardiac Glycoside Plants Self-Poisoning. *Acta Medica Bulgarica.* 2014 November; DOI: 10.2478/amb-2014-0013.
3. Seneviratne, S.L.; de Silva, C.E.; Fonseka, M.M.D.; Gunatilake, S.B.; de Silva, H.J. Post mortem findings in yellow oleander poisoning. *Sri Lanka Medical Association,* 21-24 March 2001.
4. İşıl Bavunoğlu, Musa Balta, Zeynep Türkmen; Oleander Poisoning as an Example of Self-Medication Attempt; *Balkan Med J.* 2016 Sep; 33(5): 559–562.

### Toxicology, Oleander Leaves Infusion, Fatal Intoxication



## K36 Comparison of Manual Protein Precipitation and Automated Protein Precipitation Using DPX Low Porosity Tips in Blood, Urine, and Tissues

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**Learning Overview:** After attending this presentation, attendees will understand the benefits of automating their extractions using low porosity tips in place of traditional protein precipitation methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a novel automated option for protein precipitation of various common toxicological matrices.

Historically, protein precipitation is a commonly used manual clean-up process in many toxicological drug assays for blood and tissue samples. Often referred to as “protein crash,” this initial step rapidly removes numerous biological interferences prior to a more thorough downstream sample clean up. Traditionally, an organic solvent, most commonly acetonitrile or methanol, is added to denature the sample and initiate protein precipitation. This is followed by a quick vortex step and centrifugation prior to decanting the supernatant for further extraction, if needed.

The widespread use and robustness of protein precipitation makes it an ideal process to automate, especially in laboratories with large case loads. Recently, DPX Technologies has presented an automated method for the quantitation of testosterone in serum using Low Porosity Filtration Tips (LPFT). This tip-based method uses a quick (less than two minutes) and effective, patent-pending, Tip-on-Tip (ToT) technology that automates protein precipitation and filtration to isolate the supernatant. Using the DPX tips, one can eliminate the need for manual vortex mixing, centrifugation, and supernatant transfer.

This study is a comparison of the DPX ToT method on an INTEGRA Biosciences VIAFLO using LPFT to the Orange County Crime Lab’s Scientific Working Group for Forensic Toxicology (SWGTOX) -validated method that uses a traditional protein precipitation process. The validated method analyzes for 23 drugs, including benzodiazepines, antihistamines, and sedative hypnotics, in antemortem blood, postmortem blood, urine, brain homogenate, liver homogenate, and stomach content homogenates. Brain and liver homogenates were diluted anywhere from 2x to 12x with water, and stomach content homogenates were diluted between 10x and 500x with water prior to treatment.

After completing protein precipitation, both techniques followed the validated extraction procedure. Both precipitation methods, which included standards, controls, and case samples, were injected on a Waters® ACQUITY® UPLC with a Waters® BEH C18 column (1.7µm, 2.1 x 100mm) and Xevo TQ-S to compare the results.

Various concentrations and matrices were analyzed using both techniques to determine if the ToT method using the LPFT correlated to the SWGTOX-validated method. Same-day studies between the two techniques indicated that the LPFT works just as well as the more traditional protein crash method. For antemortem and postmortem blood specimens, the percent difference between the two methods ranged between 5.8% and 8.5%. Significantly, the ToT method was able to handle not only blood and urine but also tissue homogenate samples at various pre-extraction dilutions. Varying diluted brain, liver, and stomach content specimens had % differences for the two methods of 8.3%, 4.0%, and 4.7%, respectively. Importantly, precision studies were successful using the ToT method on a concentrated liver homogenate with percent differences of 2.5% and 7.9% for alprazolam and hydroxyzine, respectively. These initial investigative results demonstrate that protein precipitation can be automated for SWGTOX-validated forensic applications for Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis. Significantly, adaptation of an automated crash method for forensic laboratories offers several unique advantages, including reducing the hands-on time requirement for the analyst, minimizing potential human errors, and further negating the long-term health effects of repeated pipetting.

### Protein Precipitation, Sample Preparation, Benzodiazepines



## K37 Variability in Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART®-HRMS) Instrument Parameter Optimization Due to Molecular Identity

Jessica L. Sprague, MS\*, Winter Park, FL 32792; Candice Bridge, PhD, National Center for Forensic Science, University of Central Florida, Orlando, FL 32816

**Learning Overview:** This presentation will introduce attendees to the importance of the identity of targeted molecules when optimizing DART®-HRMS instrument parameters. This presentation will show attendees that when developing protocols for DART®-HRMS targeted screening, the identity of the target molecule will drastically impact the performance of the instrument. Therefore, it is necessary to optimize DART®-HRMS parameters for specific compounds, rather than whole classes of molecules or evidence samples that other screening methods may target.

**Impact on the Forensic Science Community:** For DART®-HRMS instrumentation to be operationalized in a crime laboratory, instrumental methods for analysis must be optimized by the crime laboratory facility. This presentation will impact the forensic science community by emphasizing the importance of optimizing DART®-HRMS to specific compounds, which in turn improves the sensitivity of the instrument as a screening tool.

DART®-HRMS instrumentation is rising in popularity in forensic research, due to its vast applications in all forensic disciplines as a robust evidence screening tool. Recent research has shown DART®-HRMS applications in drug chemistry, toxicology, and trace evidence subdisciplines, including sexual assaults, explosives, and fire debris investigations. DART®-HRMS can be used as a faster, more specific, and more sensitive alternative to traditional forensic screening methods (i.e., Enzyme-Linked Immuno-Sorbent Assay (ELISA) and canine detection).

DART®-HRMS analysis is a highly sensitive and specific instrumental technique. Evidence samples can be introduced to the DART® ionization source in any phase of matter. Analyte molecules desorb from the surface of the sample after exposure to a heated metastable gas stream (i.e., excited-state helium). The excited metastables interact with atmospheric air molecules (i.e., water and oxygen), forming atmospheric ions. These ions cause the analyte to undergo soft ionization, resulting in the creation of molecular ions and adducts. Analyte ions are then carried through an HRMS, and the masses of the ions can be reported to four decimal places. Relative isotope abundances can then be used to preliminarily identify unknown analytes.

The instrumental parameters used in DART®-HRMS can affect the sensitivity of analysis for semi-quantitative work. Variable instrument parameters include the voltage at the exit grid, desorption temperature, and width of the sample gap, among others. Such instrument parameters can affect the ionization mechanisms at the ionization source by varying the availability and thermochemical energy of both the metastable and analyte species. Molecules that ionize most readily in DART® atmospheric conditions can dominate the resulting mass spectra, reducing the sensitivity in the analysis of other molecules in mixtures.

Optimization of DART®-HRMS parameters will be necessary for any forensic lab attempting to adopt DART®-HRMS instrumentation for casework. The preliminary data in this study notably show that the instrument parameters *must* be optimized for each compound of interest in forensic screening. Five compounds of evidentiary value were analyzed at varied exit grid voltages (50–550V, in positive or negative mode as appropriate). Each compound exhibited different functional groups and thermochemical properties, but were prepared at the same concentrations (ppm). There were no definable trends in the resulting analytical sensitivity as measured by relative standard deviations of the slope of the generated calibration curve. The results lead to the recommendation that any optimization of DART®-HRMS analyses must be performed for each specific molecule of analytical interest, rather than general molecular classes utilized by alternative screening methods.

### DART®-HRMS, Forensic Screening, Instrument Optimization



## K38 High-Throughput Screening of Drugs of Abuse in Biofluids via 96-Solid-Phase Microextraction and Transmission Mode Direct Analysis in Real Time-Mass Spectrometry (TM-DART<sup>®</sup>-MS)

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**Learning Overview:** After attending this presentation, attendees will gain insight into how DART<sup>®</sup>-MS can be automated for high-throughput screening of drugs from toxicological specimens, such as urine and plasma. Attendees will also understand how Solid-Phase Microextraction (SPME) can be automated and used to extract and concentrate the drugs of abuse from the sample matrix and reduce matrix suppression for DART<sup>®</sup>-MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing and discussing the potential value of employing SPME DART<sup>®</sup>-MS for high-throughput screening of drugs of abuse from urine and plasma. SPME provides a simple and automatable clean-up method to preconcentrate the drugs prior to analysis by DART<sup>®</sup>-MS, improving both sensitivity and selectivity. DART<sup>®</sup>-MS is an ambient ionization MS technique that has tremendous potential for high-throughput implementation due to its easy operation, speedy analysis, and high sensitivity. By coupling SPME with DART<sup>®</sup>-MS, this approach provides the forensic examiner the capability of rapidly generating high-fidelity data for the identification of drugs of abuse from urine and plasma.

Protocols for urine specimen drug tests typically involve a multi-step analysis process. First, a screening method, such as an Immunoassay (IA), is used to quickly identify specific or classes of drugs present in the urine. Next, a confirmatory method, such as Gas Chromatography/Mass Spectrometry or Liquid Chromatography/Mass Spectrometry (GC/MS or LC/MS), is used to confirm a positive or, in some instances, negative result. Although immunoassays are rapid and inexpensive, specificity varies based on the assay and can result in high rates of false positives and false negatives. Chromatography-based MS techniques can also be used as a screening tool and are very specific, but they have run times that are often lengthy, requiring up to 30 minutes per sample, which limits throughput.

To increase sample throughput while maintaining a high level of sensitivity and specificity for drug screening, liquid handling robotics was employed with SPME TM-DART<sup>®</sup>-MS to automate the SPME extraction and elution, as well as sample deposition and presentation to the DART<sup>®</sup>-MS. This coupling takes advantage of the speed and sensitivity of DART<sup>®</sup>-MS while reducing user input required for SPME and further increasing the speed of the DART<sup>®</sup>-MS analysis. Using a liquid handling robot, the manual transfer of the SPME fibers between the conditioning, extraction, and elution steps and the deposition of the eluent onto the wire mesh sample substrates for TM-DART<sup>®</sup>-MS were eliminated. With this approach, 96 samples can be analyzed in as little as 20 minutes. This approach demonstrated reduced matrix suppression and sensitivity in the parts per billion range. Bovine blood was fortified with fentanyl and detected at concentrations as low as 100ng/mL. Bovine blood was also fortified with a mixture of eight compounds, which includes fentanyl, norfentanyl, acetyl fentanyl, cis-3-methyl fentanyl, furanyl fentanyl, FIBF, 4-ANPP, and cyclopropyl fentanyl. All eight compounds were detected at concentrations as low as 100ng/mL. In addition to SPME, the liquid handling robot was also used to deposit nanoliter volumes of sample to reduce matrix suppression for TM-DART<sup>®</sup>-MS, and the resulting data showed improved sensitivity. Signal response for both methadone and codeine were significantly greater with 200nL than with 1 $\mu$ L. Furthermore, the limit of detection for methadone was improved twofold, from 100ng/mL to 50ng/mL. This approach of using SPME in combination with TM-DART<sup>®</sup>-MS and liquid handling robotics demonstrated a unique approach for high-throughput screening of drugs of abuse with good sensitivity and selectivity.

### DART<sup>®</sup>-MS, Solid-Phase Microextraction, Drugs of Abuse



## **K39 Comprehensive Analysis of 34 Fentanyl Analogs Including Carfentanil From Liver Tissue Using Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Analysis**

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**Learning Overview:** After attending this presentation, attendees will better understand a QuEChERS extraction protocol utilized for the quantitative analysis of 34 different fentanyl analogs from liver tissue and detection using an LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a comprehensive procedure for the analysis of emerging fentanyl analogs extracted from postmortem liver tissue through the use of a safe method that reduces potential contamination while maintaining sensitivity.

Opioid overdoses have increased in recent years, and according to the National Institute on Drug Abuse currently more than 130 people die from an opioid overdose each day within the United States. The analysis of these analogs from biological specimens pose an analytical challenge to practitioners due to the acute lethal intoxication concentrations, continuous variations of analogs, and wide variations in chemical structure. Additionally, Postmortem Redistribution (PMR) of concentrations of drugs such as fentanyl within a deceased individual can occur with time and has the potential to change the concentration in the peripheral blood, while studies have shown that PMR is not as frequently occurring in the liver. Therefore, based on the PMR tendencies of fentanyl, liver or other organ tissues are needed to provide the medical examiner the proper data to make an informed decision on the manner or cause of death when fentanyl is present and may provide a more adequate indication of antemortem concentrations compared to blood.

Liver specimens are often homogenized with bladed devices that can require costly disposable parts or require cleaning to avoid contamination. Here, this study is proposing to use a disposable single tube with steel beads for homogenization. Presented in this study is a QuEChERS method to extract fentanyl and fentanyl analogs from human liver samples.

This method used prepackaged extraction powder containing magnesium sulfate and sodium acetate, 0.1g of liver tissue, three stainless steel balls, and 2mL dispersive-Solid Phase Extraction (SPE) tubes, containing 25mg of Primary Secondary Amine (PSA), 25mg end-capped octadecylsilane (C18<sub>EC</sub>), and 150mg magnesium sulfate. Homogenization was achieved using a high-speed mixer mill with homemade attachments for 1.7mL centrifuge tubes and 4.5mm stainless steel balls for pulverization, reducing the risk of cross contamination by producing a homogenized sample in a single disposable tube. The quantitation method was performed on an Agilent® 6470 LC/MS/MS system coupled with a 1290 Infinity® II LC system. Chromatographic separation was achieved on a Zorbax® Eclipse Plus™ C18 RRHD 3.0x100mm, 1.8µm column with 0.1% formic acid and 5mM ammonium formate in water (mobile phase A), and 0.1% formic acid in methanol (mobile phase B).

An evaluation of a QuEChERS extraction procedure is presented in this study as an alternative analytical method for efficient extraction and detection of fentanyl and fentanyl analogs. The evaluated parameters include selectivity, matrix effects, linearity, bias, precision, and proof of applicability using authentic fentanyl case samples. Average recoveries for the 34 fentanyl analogs were 101.1% and 104.4% for low (1µg/kg) and high (50µg/kg), respectively. Average matrix effects for the 34 fentanyl analogs were 99.3% and 98.4% for low and high, respectively.

The comprehensive extraction and LC/MS/MS method developed for analysis of liver tissue for fentanyl and fentanyl analogs is precise, sensitive, and reproducible for complex forensic matrices such as liver tissue.

### **Fentanyl Analogs, QuEChERS, Postmortem Liver**





## K40 Validation and Comparison of Three Sample Preparation Techniques for Quantitation of Amobarbital, Butalbital, and Phenobarbital in Blood and Urine Using Ultra-Fast Liquid Chromatograph/Tandem Mass Spectrometry (UFLC/MS/MS)

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**Learning Overview:** After attending this presentation, attendees will be able to choose among the methods developed for the analysis of selected barbiturates in blood and urine.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on sample preparation methods for the analysis of select barbiturates in various matrices.

**Background/Introduction:** Barbiturates are a class of drugs that act as Central Nervous System (CNS) depressants. They were vastly used as sedative, hypnotic, anticonvulsant/antiepileptic, and anesthetic agents in the 20<sup>th</sup> century. Today, only 12 types of barbiturates are still in use for therapeutic purposes. The rest have been greatly replaced by benzodiazepines due to narrow margins of safety. However, barbiturates are still a prevalent choice of abuse among the public and, therefore, are of forensic toxicological importance.

**Objective:** The objectives of this research study were to validate three different sample preparation methods (Liquid-Liquid Extraction (LLE), Supported-Liquid Extraction (SLE), and Solid-Phase Extractions (SPE)) for the quantitation of three barbiturates (amobarbital, butalbital, and phenobarbital) in blood and urine using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS); and to compare the efficiency and effectiveness between methods for extraction of barbiturates under the laboratory setting at Boston University School of Medicine.

**Method:** Six-point calibration curve with three quality control samples were extracted using LLE, SLE with Biotage® ISOLUTE® SLE+ cartridge, and SPE with Waters® Oasis™ PRiME Hydrophilic-Lipophilic Balanced (HLB) cartridges. All analyses were conducted using a Waters® XBridge™ C18 LC column (3.5µm particle size; 50mm length; 2.1mm internal diameter) on a Shimadzu® UFLC coupled to a SCIEX™ 4000 QTRAP MS/MS with negative Electrospray Ionization (ESI) mode. Method validation was performed according to the American Academy of Forensic Sciences Standards Board (ASB) standard 036, First Edition 2017. Calibration model, carryover, Limit Of Detection (LOD), Limit Of Quantitation (LOQ), bias and precision, stability, dilution integrity, interferences, ion suppression/enhancement, and recovery were evaluated for quantitative analysis.

**Results:** A six-point linear calibration model (20-2,000ng/mL) with 1/x weighting was reproducible in all three sample preparation methods for quantifying amobarbital, butalbital, and phenobarbital in blood and urine with  $r^2$  greater than or equal to 0.994. Bias and precision evaluated from three controls throughout the range of the curve were within  $\pm 20\%$  and  $\pm 20\% CV$ , respectively. Neither carryover nor interferences were observed. Detection limits were evaluated down to 5ng/mL depending on the extraction procedure. Samples were able to be diluted up to 50 times prior to instrumental analysis. Samples were stable on autosampler at room temperature up to 72 hours after their initial analysis. Recovery of barbiturates from blood and urine all ranged from 45% to 86%. The effect of ionization suppression or enhancement demonstrated only minimal impact on the validation.

**Conclusion/Discussion:** This study has successfully validated three sample preparation methods (LLE, SLE, and SPE) for the quantitation of three barbiturates (amobarbital, butalbital, and phenobarbital) in blood and urine using LC/MS/MS. By comparing each method's efficiency and effectiveness, LLE was the best at quantifying barbiturates in blood and SLE was the most suitable method for extracting barbiturates from urine. These findings can be used further for examining the overall reliability and reproducibility of the validated methods in different laboratories. Results obtained can also be used to explore the possibility for streamlining sample preparation in the forensic laboratory.

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### Barbiturates, Sample Preparation, LC/MS/MS



## **K41 Determination of Synthetic Cannabinoids AB Pinaca and AB-Fubinaca With Disposable Screen Printed Carbon Electrodes (SPCE) Modified With Nanoparticles and Enzymes**

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**Learning Overview:** After attending this presentation, attendees will be able to explain the theory and use of amperometric electrochemical techniques with disposable modified electrodes as screening tools in forensic science and to understand their implications in the fast quantification of novel synthetic cannabinoids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a novel analytical approach that provides both qualitative and quantitative information. The proposed methodology allows the on-site testing and quantification of synthetic cannabinoids at low concentration without sample destruction and as a miniaturized detection system.

Characteristics linked to electrochemical analysis include the speed of analysis, low cost, sensitivity, miniaturization, and low volume of testing material, with all of them being quickly adapted to forensic sciences practice. Screen-Printed Carbon Electrodes (SPCEs) are optimal testing devices due to low cost and disposable character, small sample volume, and the possibility of hosting modification on its surface via nanomaterials or enzymes to act as biosensors. The electrochemical process taking place can provide qualitative and quantitative information about an analyte under scrutiny. Most known SPCEs are carbon-based ink, with the additional advantage of having a significant potential window for analyte detection and possible surface modifications. The development of nanostructures at the electrode surface increases the current process and facilitates the incorporation of enzymes via functionalization. Enzyme Nanoparticles (E/NPs/SPCEs) have been shown their electrochemical performance due to selectivity and sensitivity.

Four amperometric biosensors based in the inhibitory activity of synthetic cannabinoids on enzymes were developed. AB-Pinaca and AB-Fubinaca on Acetylcholinesterase (AChE) using Acetylthiocholine Iodide (ATI) and glucuronyl transferase enzyme Uridine Diphosphate (UDP) with serotonin as substrates showed their inhibitory effect on amperometric substrates current. SPCEs were modified with gold NPs (AuNPs) for AChE and Multi-Wall Carbon Nanotubes MWCNT) for UDP. Enzymes were immobilized with carbodiimide and glutaraldehyde, respectively, and experimental optimization conditions were assessed. Michaelis-Menten apparent constants (KMapps) of biosensors were estimated in the presence and absence of inhibitors. Increasing slopes of calibration curves and values of KMapps indicate inhibitory effect at low concentration. Biosensors showed Limit of Detection (LOD)  $(8.4-13.0) \times 10^{-5}$  M; Limit of Quantitation (LOQ)  $(2.9-4.3) \times 10^{-4}$  M; Relative Standard Deviation (RSD) repeatability slopes (3.4–8.2%); RSD reproducibility slopes (3.6–9.7)%, and recovery (96.8–99.6)%. This is the first time that biosensors based on the inhibitory effect of synthetic cannabinoids on these enzymes have been applied to quantitative determination.

**AB Pinaca Fubinaca, Disposable Electrodes, Enzymes Nanoparticles**

## K42 A Fatal Case of Body Stuffer Syndrome

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**Learning Overview:** This case of a fatal cocaine overdose in a body stuffer not only confirms the limits of instrumental diagnostics in the recognition of corporal drug users, but also helps to better define the pathological characteristics of this peculiar syndrome. This case also demonstrates that cocaine body stuffers, contrary to general belief, can develop toxic symptoms beyond 24 hours after ingestion, and that delayed toxicity can occasionally lead to death.

**Impact on the Forensic Science Community:** This case will impact the forensic science community by better defining histopathological and toxicological findings of the unique type of cocaine overdose known as “body stuffer syndrome.”

**Introduction:** Intracorporeal drug concealment is a frequent illicit practice in drug trafficking, since it is impossible to identify drug carriers if no medical complications occur.<sup>1,2</sup> Sometimes, drug concealment is the impulsive act of consumers or small traffickers (the so-called mini packers or body stuffers) attempting to evade control by law enforcement. In this case, the risk of acute intoxication is higher, because poor drug packaging, due to the extemporaneousness of the gesture, results in an increased probability of rupture or leakage.<sup>3,4</sup> The clinical picture, known as “body stuffer syndrome,” varies according to the type of drug and the amount of substance absorbed, and can prove lethal, as exemplified in this case.<sup>5-8</sup>

**Case Report:** A 29 year-old female drug pusher, while in custody, reported general malaise with abdominal pain. Physical examination, conducted at the local hospital’s emergency department, revealed: hypothermia, tachycardia, hypotension, mydriasis, areflexia in the lower limbs and hyporeflexia in the upper limbs, right supra-orbital hematoma, and right shoulder hematoma. Electrocardiogram (ECG) only confirmed tachycardia, while echocardiogram did not show abnormalities. A total body Computed Tomography (CT) scan revealed: peri-ponto-mesencephalic subarachnoid hemorrhage, ischemic brain lesions, and hepatic injury with perihepatic and perisplenic fluid effusion (without splenic lesions). Blood analysis showed hyperglycemia, leucocytosis, high transaminase levels, and hypercapnic acidosis. Toxicological tests revealed high levels of cocaine and its metabolite benzoylecgonine in serum (over 50.00g/L and 5.26 /L, respectively) and urine (22.5g/L and 76.9g/L, respectively). The patient rapidly went into a coma; she was admitted to the Intensive Care Unit (ICU) and placed under mechanical ventilation and colloid infusion for hypotension. She died three days after admission. At autopsy, a single, open plastic bag, undetected by the previous CT scan, was found in the gastric lumen, which presented several deep, necrotic ulcers. Hemorrhagic areas in the lungs, kidneys, and liver, and a (modest) peri-ponto-mesencephalic leptomenigeal hemorrhage were also observed. The heart showed mild concentric myocardial hypertrophy (ventricular wall maximum thickness =2.2cm), and a small hemorrhagic area in the anterior papillary muscle. Histological examination confirmed the polyvisceral bleeding pattern; particularly relevant were the plentiful hepatic hemorrhagic foci, communicating with dilated sinusoids, in complete absence of inflammatory reaction. Heart samples showed microarterial damage and foci of contraction bands; deep ulcerations, bounded by parietal necrosis, and intense hemorrhagic phenomena were detected in the gastric samples. Postmortem toxicological analysis documented very high levels of cocaine and benzoylecgonine in all biological samples (5.74g/L and 43.23g/L in urine, 1.72g/L and 4.3 g/L in blood, respectively) and cocaine in the plastic bag content (381.20g/L).

**Discussion:** Cocaine is the drug most commonly involved in severe intoxications associated with body concealment, although fatal cases in body stuffers are rare, according to literature, in comparison with heroin, cannabis, and ecstasy toxicosis.<sup>9,10</sup> This case of fatal cocaine overdose in a body stuffer not only confirms the limits of instrumental diagnostics in the recognition of corporal drug users, but also helps to better define the pathological characteristics of this peculiar syndrome. In fact, to date, conventional radiology—normally used to search for drug packages within the gastrointestinal system—have limited diagnostic value, as exemplified by this case in which the radiological images conducted have been thoroughly revised, with a negative outcome. The case presented here also demonstrates that cocaine body stuffers can develop toxic symptoms beyond 24 hours after ingestion, and that delayed toxicity can, occasionally, lead to death.<sup>11,12</sup> It appears that the integrity of the packaging has a significant predictive value in determining the time and likelihood of toxicity, although in some fatal cases the autopsy showed intact packages.<sup>13</sup> In this case, sustained leakage from the package into the gastric lumen probably led to a massive absorption of cocaine into systemic circulation, causing a dramatic clinical picture and the lesions observed at autopsy and at histological examinations, of which the “peliotic-like” liver alterations (dilation of the hepatic sinusoids, communicating with cystic blood cavities, delimited by endothelium, and surrounded by a band of connective tissue) were the most atypical, for liver damage is not usually reported in cocaine intoxications.<sup>14-18</sup>

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### **Body Stuffer Syndrome, Forensic Histopathology, Forensic Toxicology**

## K43 A Different Approach in the Estimation of the Time Since Death: Concurrence of Thanatochronological and Toxicological Data in a Case of Cocaine Assumption-Related Death

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**Learning Overview:** The goal of this presentation is to provide a different approach in the evaluation of the Postmortem Interval (PMI).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by giving an example of how toxicology and thanatochronology could be integrated in the estimation of the time since death.

**Introduction:** The estimation of the time since death has been a longstanding issue for forensic pathologists. Its usefulness is mostly evident in cases of violent death, in which a precise evaluation of the PMI may help direct investigation enquiries to a potential suspect of homicide. Furthermore, it can sometimes be relevant in case of other crimes, such as failure to provide assistance.

As is widely known, the parameters obtained through the evaluation of supravital reactions, which are usually used to establish the PMI, are modified by variation of numerous factors related to the environment and to the body itself.<sup>1,2</sup> Because of this variability, it is not always easy to obtain a precise chronological reference of death, despite the technologic progress and the introduction of new methods, such as the biochemical analysis of vitreous humor and the study of electrical excitability of skeletal muscle; furthermore, often only a wide time window is obtained.<sup>3-5</sup> However, the estimation of the decrease of body temperature is still the most reliable method used, supplemented by the others thanatochronological criteria.<sup>6</sup>

In some cases, toxicology can help pathologists to better define the PMI; in fact, when the time of consumption is known, it is possible to estimate the time elapsed since the consumption of a substance by its concentration in blood and tissues.<sup>7</sup>

This work is about a case of cocaine-related-death, in which the definition of a precise PMI, obtained by the concurrence of thanatochronological and toxicological data, had a fundamental role in substantiating the failure-to-provide-assistance hypothesis.

**The Case:** A 47-year-old man was found dead at 6:30 p.m. in a hotel room, where he arrived with his girlfriend around 3:30 a.m.–4:00 a.m. of the same day. The woman reported the man took intravenous cocaine first around 1:00 a.m. when they were in another place, and then a second time in the hotel room, approximately between 4:00 a.m.–5:30 a.m. During the first interrogation performed by the police, she said she left the room at 5:30 a.m. while the man was sleeping; then she said she went back to the hotel around 12.00 a.m. to bring him home, but he preferred to stay, so she left again and never came back. This reconstruction of the facts was denied by the hotel keeper's witness, who reported that the woman definitely left the room around 12:00 a.m. At this point, the woman gave another version of the story; she said she left the hotel at 1:00 p.m. and her boyfriend was still alive.

The judicial authority supposed the man had died in a timeframe incompatible with the woman's story, so the hypothesis of failure-to-provide assistance took place. The thanatochronological data (obtained during the inspection at 8:30 p.m.: body temperature 31.0°C/87.8°F, room temperature 19.3°C/66.74°F) placed the time of death around 9:30 a.m.; the Henssge nomogram (corrective factor: 1) was used. The autopsy didn't reveal any concomitant disease or traumatic lesions able to justify the death, except for a sign of needle puncture at the right groin.

To assess the cause of death, toxicological analyses were performed and the cocaine and benzoylecgonine levels in the blood (446ng/ml and 8,427ng/ml, respectively), brain (0.51ng/mg and 1.56ng/mg, respectively), and liver (0.05ng/mg and 3.48ng/mg, respectively) confirmed cocaine overdose as the cause of death. The toxicological data were also used to estimate the PMI. The blood, brain, and liver cocaine/benzoylecgonine ratio (0.05, 0.33 and 0.014, respectively) and the brain/blood cocaine ratio (1.14) placed the death around 4–6 hours after cocaine injection. This chronological reference perfectly matched with thanatochronological information.

This case represents an example of how the classic thanatochronological data could be integrated with toxicological analysis to estimate the time since death, if the time since the last intake of the substance of interest is known. This could be a different approach for solving the historical issue of the evaluation of PMI.

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### Time Since Death, Postmortem Toxicology, Cocaine Overdose

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## K44 Determination of Nicotine and Cotinine in Saliva

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**Learning Overview:** It is important to determine the amount of nicotine and cotinine in oral fluids by instrumental methods in body fluids. It is crucial to have confirmation of the declarations of the employees that they are not smokers. Thus, the goal of this study was to determine the fast and accurate chromatographic methods for the determination of nicotine and cotinine in oral fluids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that there are several methods for determining nicotine and cotinine in saliva.

The level of nicotine and cotinine increases in the body depending on the frequency and amount of smoking. With increased use of cigarettes, an increase of these substances is observed in the saliva, which is an important sample in forensic sciences. Regardless of the use of harmful products, it is important to determine compounds in the body fluids. Measurement in a short time will, of course, shorten the time for instant determination, but it is necessary to use instrumental analyses to make quantitative determinations and to detect very small concentrations in the body. A few of these are High-Performance Liquid Chromatography (HPLC), Gas Chromatography/Mass Spectrometry (GC/MS), and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). An ideal marker for assessing the smoking status of individuals should have a reasonable half-life, be specific, and be obtained at concentrations that can be measured using available analytical methods.<sup>1</sup> Nicotine has an important place for tobacco addiction among cigarette smoke components. The half-life of nicotine is approximately two hours, and the duration of analysis in saliva should be adjusted accordingly.<sup>1</sup> Cotinine is the main metabolite of nicotine and it is specific. The half-life of cotinine is approximately 18–20 hours. It is a preferred analyte because it meets the necessary conditions and can be detected in all matrices.<sup>1</sup>

Samples were taken from 50 smoking and 50 non-smoking volunteers from a dental clinic in Istanbul for analysis. For example, saliva samples that are held in the mouth for five minutes were used (at this stage, the main purpose is avoiding foaming). Three different methods were determined for saliva intake. In the first method, a paper pad (1mL holding capacity) saturated with sodium chloride (3.5%), citric acid (0.3%), potassium sorbate, sodium benzoate, and gelatin (0.1% each) was used.

The pH was adjusted to 7.2 by addition of sodium hydroxide. The treated pad was dried, placed in the mouth for about two minutes, and gently moved back and forth to ensure it was moistened. Samples were centrifuged twice for 10 to 15 minutes at 10,000rpm to prevent residues or non-food particulate matter from mixing in the recovered saliva. The clear saliva sample thus obtained was frozen at 20°C for analysis.<sup>1</sup> The other method was to collect the sample with the help of Salivette® tube.

The volunteer was asked to chew the cotton taken from the inner tube for two minutes until it was saturated with saliva. The cotton was taken back to the inner tube, centrifuged, and the saliva sample was passed to the outer tube. Another method was to collect saliva passively and keep the samples immediately between 2°C–8°C, then store it at -80°C until analysis.<sup>2</sup> The most important goal is to achieve fast, reliable reporting in forensic cases with various techniques to measure nicotine and cotinine levels in the oral matrix, including colorimetric experiments, immunological tests, saliva tests, GC, and HPLC.<sup>3</sup> The methodology of this study is based on GC/MS.

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### Instrumental Analysis, Cotinine, Nicotine

## K45 The Detection and Quantification of Fentanyl in *Phormia Regina* (Calliphoridae) and Its Effects on Growth and Developmental Rate

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**Learning Overview:** After attending the presentation, attendees will understand the detection of fentanyl in black blow fly (*Phormia regina*) (Meigen) larvae using Supported Liquid Extraction (SLE) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Attendees will also learn how the presence of fentanyl in the food substrate of larvae affects their growth and development throughout larval stages.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing entomological data on *Phormia regina* to increase accuracy of minimum Postmortem Interval (mPMI) determinations as well as provide a quick method of extraction and detection for fentanyl in blow fly larvae.

As a primary colonizer of carrion, *Phormia regina* plays a vital role in both nutrient recycling and ecosystems via carrion decomposition. They are currently used by forensic scientists in establishing mPMI estimation during criminal cases, but recent research has that larvae can be further used for the detection of drugs and toxins when traditional matrices are unavailable.<sup>1,2</sup>

Fentanyl is a rapid-onset synthetic opioid with extremely high potency.<sup>3</sup> Illegally, fentanyl has been increasingly used as an adulterant mainly due to the ease of manufacturing and availability of its precursors to be shipped internationally.<sup>4</sup> Due to the increase in accidental deaths by overdose, fentanyl is now routinely screened for in toxicological analysis. Death from fentanyl can be rapid, and by the time a body is discovered, significant time may have passed. When sampling traditional body fluids becomes difficult due to their absence, external factors, or the presence of skeletonization, insects may serve as reliable alternative specimens for toxicological analyses.<sup>1</sup>

There have been several studies that have confirmed the reliability of entomological specimens for qualitative analysis; however, quantitative extracts remain questionable and unreliable. In addition, the presence of toxins has been shown to increase or delay development of entomological specimens, which can be critical in a criminal investigation. It has become critical to understand the detection and effects of fentanyl on different matrices due to its increased use and high contribution to overdosing.

The purpose of this study was to further analyze the question currently poised for forensic entomologists/toxicologists: is there is any correlation between concentrations of toxins in human postmortem tissue to those concentrations detected in blow fly larvae and can they be used reliably as samples for toxicological investigations? Using *Phormia regina*, this study evaluated LC/MS/MS to quantify and qualify fentanyl accurately as well as obtain some preliminary information pertaining to the effects of fentanyl on larvae development.

A maximum of 100 individual pupae received from the University of New Haven in West Haven, CT, and University College of St. Mary in Omaha, NE, were placed inside a mesh enclosure with an ambient temperature of 26–28°C. Chunks of beef liver were provided to the adults to facilitate oviposition. Eggs were collected and placed on homogenized ground liver pre-dosed with fentanyl. The doses were a high-end therapeutic level (50ng/mL), a toxic level (125ng/mL), a lethal level (250ng/mL), and beyond lethal level (500ng/mL).

The larvae were monitored, and sample larvae were taken every day to confirm the lifecycle stage. Triplicate larvae samples were collected at the 2<sup>nd</sup> instar, 3<sup>rd</sup> instar, and post-feeding life stages. The larvae were homogenized, extracted, and analyzed using LC/MS/MS. Fifteen random specimens were selected and used as a representative set every day for each dose to monitor life stage duration. These larvae were also measured to obtain a growth curve.

During the trial, fentanyl was detected in 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae fed on toxic and lethal level dosed meat while detection remained minimal or nonexistent in the post-feeding larvae of any dose. There was no correlation found between the feeding substrate and the larvae samples of any stage of the life cycle. The development rate to 2<sup>nd</sup> instar was delayed and correlated with the increase of fentanyl, thus prolonging the total time for development from two to four days.

With the increase of fentanyl throughout the Eastern coast of the United States and the prominent presence of *Phormia regina* in decomposition, this study aimed to find results that will lead to the use of larvae as alternative matrices. This study demonstrated that fentanyl was detectable in 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae in toxic and lethal doses. Further, it demonstrated that fentanyl delayed development in *Phormia regina* by up to four days when exposed to high levels of lethal dosing, effecting accurate PMI. It was also concluded that there is no correlation between spiked food substrates and the concentration found in the analyzed insect.

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### Entomotoxicology, Fentanyl, *Phormia Regina*

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## K46 Emergence of Delta-8 Tetrahydrocannabinol (THC) in Driving Under the Influence of Drugs (DUID) Investigation Casework

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**Learning Overview:** After attending this presentation, attendees will be able to identify delta-8 THC and its appearance in various marijuana-containing products. Additionally, this presentation will illustrate an important analytical challenge related to constantly changing drug trends and how it was addressed in a high-throughput laboratory setting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the recent increase in prevalence of THC isomers in DUID cases. Furthermore, an example method is described, along with its validation and application, showing how it effectively reduced both repeat testing and cancellations due to unresolved peaks.

Cannabinoids are the most frequently reported illicit drug class in DUID investigation casework, with a consistent positivity rate over 50%. Since September of last year, the cancellation rate for the cannabinoids confirmation performed using a 2D High-Performance Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) demonstrated a steady increase (from 1.3% to 3.8%) due to the presence of unresolved interfering substances. The observed interference demonstrates a distinctive pattern affecting identifications and quantification of both delta-9 THC and delta-9 Carboxy THC (THCCOOH).

As a part of an investigation, an e-cigarette device containing an amber-colored viscous solution was submitted to the laboratory. The oily substance was diluted 100-fold for analysis. Two peaks with equally high response were identified as delta-9 THC and delta-8 THC, in addition to a presence of Cannabidiol (CBD) and four other unidentified components in the e-cigarette cartridge. Ultimately, additional work revealed delta-8 THC ( $RT=5.01\text{min}$ ) and delta-8 THCCOOH ( $RT=3.05\text{min}$ ) to be responsible for the observed interferences with delta-9 THC ( $RT=4.92\text{min}$ ) and delta-9 THCCOOH ( $RT=3.14\text{min}$ ), respectively.

Delta-9 THC and delta-8 THC are constitutional isomers ( $C_{21}H_{30}O_2$ ; MW 314.46), as are their carboxy metabolites ( $C_{21}H_{28}O_4$ ; MW 344.44). The similarity between the two isomers was not previously a challenge due to the relatively small concentrations of delta-8 THC encountered after traditional marijuana consumption. Delta-8 THC is a naturally occurring cannabinoid found in most cannabis plants. It is typically present at low concentrations (less than 1% of dried cannabis flowers). It is psychoactive, but has a lower potency as compared to delta-9 THC. Recently, delta-8 THC has been marketed as an alternative to marijuana. Delta-8 THC has been identified in botanicals, e-liquids intended for vaping, and edibles. The increase in cancellations due to delta-8 compounds required modification of the existing method that had been successfully employed for the past three-and-a-half years.

To separate the delta-8 and delta-9 isomers, a new analytical method was developed and validated. Cannabinoids were extracted from whole blood using liquid-liquid extraction, separated in a 2D LC system with a Phenomenex® SecurityGuard™ Cartridge (C6-Phenyl, 4 X 2mm) as a trap column and a Phenomenex® Kinetex® C18 (100 x 3mm; 2.6micron) as an analytical column, over a runtime of 11.5min and detected by an AB SCIEX™ API 4500 system equipped with an Electrospray Ionization (ESI) source operating in positive ionization mode with scheduled multiple reaction mass spectrometric monitoring. The method validation protocol was based on the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines, including linearity, limit of detection, lower limit of quantitation, precision and accuracy, interfering substances, matrix effect, dilution of samples, and carryover. The calibration for THC and THCCOOH was linear from 0.5 to 50ng/mL and 5.0 to 500ng/mL, respectively. The minimum total precision (described as %CV) and accuracy (shown in %difference) were 3.4% and -3.4% for THC and 3.1% and -3.9% for THCCOOH, respectively. The method met validation criteria for all studies. Most importantly, each set of isomers were fully resolved: delta-8 THCCOOH ( $RT=5.69\text{min}$ ); delta-9 THCCOOH ( $RT=5.74\text{min}$ ), delta-9 THC ( $RT=8.21\text{min}$ ); and delta-8 THC ( $RT=8.65\text{min}$ ).

For efficiency, routine DUID cases continue to reflex to the pre-existing 2D LC/MS/MS cannabinoids confirmatory analysis with a 6min runtime. However, if the presence of delta-8 isomers is indicated, the blood specimen is re-analyzed to separate the cannabinoid isomers using the new method. With this new strategy, the cancellation rate decreased from 3.8% to 0.6%, providing forensically defensible results in more than 100 cases.

### Delta-8 THC, Isomers, Vaping





## K47 A Seven-Year Review of Vehicular Crash Toxicology Data at the West Tennessee Regional Forensic Center

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**Learning Overview:** The goals of this presentation are to: (1) describe trends of alcohol and drug use in vehicular fatalities at the West Tennessee Regional Forensic Center from 2011 to 2017; and (2) identify variables that may be related to vehicular substance use, such as age, gender, race, and crash type.

**Impact on the Forensic Science Community:** Drug-related vehicular fatalities remain an important public safety issue. This research provides a preliminary assessment of toxicology data for vehicular fatalities in Shelby County, TN. The hope is to provide direction for future research on postmortem toxicology.

According to 2016 data from the Governors Highway Safety Association, 43.6% of nationwide motor vehicle fatalities tested positive for at least one drug.<sup>1</sup> The Centers for Disease Control (CDC) show that alcohol was involved in 28% of motor vehicle fatalities in 2016, while other illicit drugs were implicated in 16% of fatalities.<sup>2</sup> Recent data indicate that drug-related motor vehicle crashes remain an important public safety issue.<sup>1,2</sup> The purpose of this work is to describe the trends of alcohol and drug use in motor vehicle fatalities at the West Tennessee Regional Forensic Center (WTRFC) from 2011 to 2017.

Information was extracted from the Forensic Death Investigation and Decedent Information (ForensicDIDI) database for all fatal vehicular crashes in Shelby County, TN, from 2011 to 2017. A total of 1,150 fatal vehicle crashes were reported. Of these, 717 (62%) decedents had toxicology data available. For the 717 decedents with toxicology data, 412 (57.5%) tested positive for at least one substance, while 305 (42.5%) tested negative for any substance. Drugs were consolidated into six classes and analyzed for trends of use from 2011 to 2017.

Analysis revealed that 78% of decedents with positive toxicology results were male. Decedents were 46% White, 49% Black, and 5% other races. Close to half of decedents (48%) were between the ages of 18 and 34 years. Polysubstance use was noted among 44% of decedents, with the remaining 56% of decedents testing positive for a single substance. A majority of decedents (74%) were drivers, while 26% were passengers, pedestrians, or unknown. Single vehicle crashes (54%) were slightly more common than multiple vehicle crashes (45%). Alcohol (38%) was the most commonly detected drug, followed by marijuana (28%) and opioids (13%). Cocaine (8%) and benzodiazepines (7%) were detected in a similar number of decedents, while amphetamines (3%) and methamphetamine (3%) were found in the lowest numbers. Overall, drug use remained steady with a slight increase in substance use during the 2015–2017 period. The most notable increase was marijuana, particularly in the years 2016–2017. Amphetamine use has shown a decline, while methamphetamine use has slightly increased. Seventy-five percent of decedents who tested positive for alcohol were at or above the legal limit Blood Alcohol Concentration (BAC) of 0.08. Furthermore, 67% of decedents had a BAC between 0.1 and 0.3. In terms of polysubstance use, 44% of decedents tested positive for two or more drugs. Polysubstance use increased during 2015–2017, with the years 2016 and 2017 showing an increase in polysubstance use that closely parallels the number of single-substance users. For the two most common drugs, alcohol and marijuana, 76 decedents tested positive for both substances, which equates to 43% of marijuana users and 31% of alcohol users.

This review of toxicology data at the WTRFC serves as an initial look at the prevalence of drug- and alcohol-related motor vehicle fatalities in Shelby County. Decedents who tested positive for one or more drugs were overwhelmingly male and nearly half of decedents were in the 18-to-34-year-old age range. Alcohol, marijuana, and opioids were the most common drugs implicated in fatalities, with alcohol being the most prevalent. Marijuana use showed a notable increase and was consistently the second most common substance detected. A sizeable percentage (44%) of decedents were found to be positive for two or more substances, pointing to polysubstance use while driving as a common issue. Future research in this area could work to better characterize postmortem drug levels that indicate intoxication.

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### Vehicular Fatalities, Toxicology, Forensic Pathology



## K48 Toxicological Findings and Demographics of Phencyclidine (PCP) Use in Houston From 2013 to 2018

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**Learning Overview:** After attending this presentation, attendees will have learned about the prevalence, blood toxicological profile, and demographic distributions of PCP-positive Houstonian drivers involved in a traffic accident or suspected of impaired driving. Attendees will also learn about common signs and symptoms observed during Drug Recognition Expert (DRE) evaluation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing valuable regional information to better understand the demographic patterns of PCP-impaired or suspected impaired drivers in Houston, TX, over six years. The long-term objective is to help design and implement regulations and prevention systems that will lead to a reduction in PCP-impaired driving.

The first reported illicit use of PCP occurred in San Francisco, CA, in the 1960s. Shortly thereafter, PCP use was found in other metropolises, including Chicago, IL, Miami, FL, New York, NY, and Philadelphia, PA. While abuse of PCP subsequently declined at the national level, it continues in Houston. In 2010, the Drug Enforcement Administration (DEA) reported the arrest of nine Houston residents and the seizure of 57 gallons of PCP, the largest PCP seizure in DEA history.

This research evaluates PCP-positive cases of driving while intoxicated or driving under the influence of drugs, occurring from 2013 to 2018. The blood samples were collected from drivers and submitted by the Houston Police Department (HPD). They were then analyzed for alcohol and drugs by the HPD Crime Laboratory, Reference Laboratories, or Houston Forensic Science Center (HFSC). Toxicological findings and demographic information, including age, sex, and race/ethnicity, were evaluated for the impaired driving cases that tested positive for PCP in blood. Additionally, the Drug Influence Evaluation findings completed by DRE officers for selected cases in 2018 were examined when the officers deemed the individuals to be under the influence of a dissociative anesthetic. IBM® SPSS Statistics version 24 and Microsoft® Excel® 2016 were used for statistical evaluation.

A total of 610 Driving While Intoxicated (DWI) cases positive for PCP in blood were identified in which the traffic offense occurred between August 2013 and December 2018; they represented approximately 16% of all DWI cases from 2013 to 2018 (estimated  $n=3,929$ ). The mean (median, range) PCP concentration was 47 (43, 7-180) ng/mL. Twenty-two percent of those cases were female and 78% were male; 85% were Black, 10% were White, and 5% were other races/ethnicities as identified by the arresting officer. No significant differences in median and distribution of PCP concentrations ( $P's > 0.05$ ) were observed between females (median 43ng/mL) and males (43ng/mL); among Blacks (44ng/mL), Whites (39ng/mL), and others (37ng/mL); and among offense years, 2013–2018 (36-58ng/mL). Forty three percent of the cases were positive for PCP only. Among the remaining 57% in which one or more other drugs/metabolites were identified, cannabinoids were the most frequently detected analytes (35%), followed by ethanol (13%) and cocaine/metabolite (13%). When the study population was examined by age ( $\leq 30$ , 31–40, and  $\geq 41$  years), the proportions of male and Black PCP-positive drivers decreased with younger age groups. For 12 cases from 2018, signs and symptoms of PCP-positive drivers observed by DRE officers were examined. Common indications included slurred speech, chemical breath odor, watery and/or bloodshot eyes, vertical/horizontal gaze nystagmus, impaired coordination/balance, and impaired divided attention.

Median PCP concentrations among demographic cohorts were remarkably similar, which indicates a possible concentration range desired by PCP users. The PCP concentration range found in the present study were comparable to other reports (7–240ng/mL), albeit the mean value in the present study was slightly lower.<sup>1-3</sup> The number of PCP-positive DWI cases has been rising in Houston in recent years (166% increase from 2016 to 2018), making risk assessment of PCP-impaired driving for evaluation efforts increasingly important.

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3. Jones et al. *Long-term phencyclidine (PCP) usage trends in the District of Columbia*. The Society of Forensic Toxicologists 2018 Annual Meeting.

**PCP, Toxicology, Blood**



## **K49 The Prevalence and Crash Risk of Drugs in Injured Drivers in Victoria, Australia**

*Dimitri Gerostamoulos\*, Southbank, Victoria, AUSTRALIA*

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**WITHDRAWN**



## K50 The Effects of Synthetic Cannabinoids and Poly-Drug Use on Drug Recognition Expert Evaluations

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**Learning Overview:** After attending this presentation, attendees will be able to describe the effects of synthetic cannabinoids, both unaccompanied and in combination with other drugs of abuse, such as fentanyl, cocaine, or methamphetamine, on evaluations performed by Drug Recognition Experts (DREs).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing data related to the physiological signs and symptoms associated with the ingestion of synthetic cannabinoids, both on their own and in combination with other commonly encountered drugs in oral fluid collected concurrently with evaluations performed by DRE officers.

The DRE program was developed to establish a systematic procedure for identifying and documenting signs of impairment in subjects suspected of being under the influence of drugs. The DRE program is currently the best tool for evaluating a subject's drug-related impairment at the roadside. Several studies have demonstrated the reliability of the DRE program for traditional drugs of abuse; however, there are limited reports related to the constellation of symptoms associated with use of synthetic cannabinoids alone or in combination with traditional drugs of abuse. The purpose of this evaluation was to compile DRE evaluation data and relate it to the analytical findings obtained from comprehensive oral fluid testing.

Participation in the evaluation was strictly voluntary and performed as part of the DRE certification process. A total of 25 subjects were assessed using the standardized evaluation protocol for drug influence. Parameters of evaluation included presence or absence of nystagmus, pulse rate, Lack Of Convergence (LOC), One Leg Stand (OLS), Walk And Turn (WAT), modified Romberg balance, pupil size in three different lighting conditions, blood pressure, and body temperature, among others. At the conclusion of the evaluation, an oral fluid sample was collected using the Immunalysis™ Quantisal® device for laboratory-based analysis. Samples were analyzed using a SCIEX™ TripleTOF® 5600+ quadrupole time-of-flight mass spectrometer coupled to a Shimadzu® Nexera® ultra high-performance liquid chromatograph.

Five subjects tested positive for 5F-MDMB-PICA, a popular synthetic cannabinoid, in the absence of any other drugs. In all five cases, no nystagmus was noted, and only one had slight indications of LOC. All subjects had difficulty with the WAT test (e.g., missing all heel-to-toe steps, inability to keep balance, and stepping off the line) and OLS test (e.g., swaying while balancing, using their arms, hopping, and putting their foot down). Only one of five of the DRE opinions included cannabis.

Fifteen subjects tested positive for fentanyl in combination with 5F-MDMB-PICA, with 4 of the 15 also testing positive for 4F-MDMB-BINACA. Horizontal Gaze Nystagmus (HGN) and a lack of smooth pursuit were noted in only one individual. LOC was present in ten of the subjects (66%) and most exhibited flaccid muscle tone. All 15 DRE opinions correctly noted a narcotic analgesic, but only 2 also indicated cannabis.

Three subjects tested positive for cocaine and 5F-MDMB-PICA, and two subjects tested positive for methamphetamine and 5F-MDMB-PICA. One individual displayed HGN and a lack of smooth pursuit. All five subjects demonstrated difficulty on the WAT and OLS tests; four subjects had LOC. Only one subject had dilated pupils. The DRE opinion in all five cases was a Central Nervous System (CNS) stimulant.

The opinion of a DRE is based on physiological and behavioral indicators consistent with specific drug categories. The ever-changing drug market, which now includes combinations of Novel Psychoactive Substances (NPS) -like synthetic cannabinoids as well as an increase in the frequency of poly-drug use encompassing NPS and traditional drugs of abuse, adds to the complexity of the DRE evaluation. In cases in which fentanyl is present, physiological signs and symptoms of other drugs used could be masked. Therefore, comprehensive toxicology testing, including NPS, is imperative to ensure all substances contributing to the impairment are identified.

### Synthetic Cannabinoids, Fentanyl, DRE



## **K51 The National Forensic Laboratory Information System (NFLIS) Survey Findings: Toxicology Testing Practices by Toxicology Laboratories and Medical Examiner and Coroner Offices**

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**Learning Overview:** After attending this presentation, attendees will understand the findings from the Drug Enforcement Administration's (DEA's) 2017 NFLIS Survey that were not previously published.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing toxicology laboratories and medical examiner, and coroner offices information regarding toxicology testing practices for opiates, opioids, amphetamines, cocaine, marijuana/Tetrahydrocannabinol (THC), phencyclidine, phenethylamines, piperazines, synthetic cannabinoids, and synthetic cathinones. This presentation will also report on respondents' use of toxicology reference laboratories.

The NFLIS is a program of the Drug Enforcement Administration (DEA), Diversion Control Division. DEA conducted a 2017 NFLIS Medical Examiner and Coroner (MEC) Office Survey and a Toxicology Laboratory (TL) Survey between June and October of 2017. These results were published on the NFLIS website. Survey results highlighted findings related to operation, caseloads, turnaround times, toxicology testing practices, accreditation, and information management systems. The purpose of this presentation is to provide the community with information on toxicology testing practices that were not reported in these publications. This information can benefit laboratory management decisions as well as aid interpretation of national statistics.

MEC and TL respondents were asked to report their testing frequency as always, sometimes, or never for specific drugs and drug classes. Further, they were asked to report their frequency of quantifying these drugs and drug classes using the same measures. Data from TLs are reported by laboratory ownership (public or private) and by caseload size of the responding laboratory. Data from MECs are reported by type of office and jurisdiction size if reported by the responding MEC.

TLs reported "always" conducting toxicology testing for the following drugs or drug classes more than 75% of the time: alcohol, amphetamines, barbiturates, benzodiazepines, carisoprodol, cocaine, heroin, marijuana, opiates and opioids, phencyclidine, and Z-drugs (e.g., zolpidem). TLs reported opiates or opioids other than heroin or fentanyl as the least likely drug class to never be quantified (11%), whereas fentanyl-related substances were most likely to never be quantified (52%). Higher percentages of small and public TLs (both greater than 30%) than medium, large, and private TLs (each less than 25%) reported never quantifying fentanyl.

MECs reported "always" conducting toxicology testing for the following drugs or drug classes more than 75% of the time: alcohol, amphetamines, cocaine, and opiates or opioids other than heroin and fentanyl. Overall, less than 13% of MECs reported never quantifying buprenorphine, fentanyl, fentanyl-related substances, heroin, and opiates or opioids other than heroin or fentanyl.

NFLIS publicly shares data through various reports throughout the year, including midyear and annual reports. As NFLIS expands into other data collections such as toxicology laboratory data, additional reports such as on the toxicology laboratory survey will be provided to the community.

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### **NFLIS, DEA, Toxicology**



## K52 Data-Supported Poly-Drug Use Among Fentanyl Users: A Toxicology Perspective

Alex J. Krotulski, MS\*, Center for Forensic Science Research & Education, Willow Grove, PA 19090; Susan Varnum, PhD, Temple University, Philadelphia, PA ; Barry K. Logan, PhD, NMS Labs, Willow Grove, PA 19090

**Learning Overview:** After attending this presentation, attendees will be able to discuss the extent to which fentanyl drug users are consuming other substances, including legacy drugs of abuse and Novel Psychoactive Substances (NPS), and the drug classes that are most commonly found in combination with fentanyl.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing comprehensive data regarding patterns and practice of poly-drug use that, in turn, have an impact on policies relating to death investigation and forensic toxicology testing practices, as well as public health and public safety preparedness and response.

The opioid epidemic continues to contribute to morbidity and mortality in the United States, growing and evolving since the increase in prevalence of fentanyl in the heroin supply began around 2014. Following the identification of mixtures of fentanyl and heroin in seized material, laboratories began identifying new variants of fentanyl, often referred to as analogs or derivatives. The number of new fentanyl analogs in the drug supply increased and diversified until the temporary federal “core structure” scheduling of fentanyl-related substances by the Drug Enforcement Administration in February 2018. Now, post fentanyl analog scheduling, the illicit synthetic opioid market has transitioned back to primarily fentanyl, but with evidence of poly-drug use. Based on this observation, this study sought to document patterns of poly-drug use in forensic toxicology casework to determine what substances were most frequently found in conjunction with fentanyl.

Analytical testing was performed via Liquid Chromatography quadrupole Time-Of-Flight Mass Spectrometry (LC/qTOF/MS) using a SCIEX™ TripleTOF™ 5600+ coupled with a Shimadzu® Nexera® XR UHPLC. This represents a non-targeted drug testing approach that differs from traditional forensic toxicology testing protocols. Discarded sample vial extracts, primarily collected from testing procedures for the directed analysis of synthetic opioids, were acquired from a large forensic toxicology laboratory (NMS Labs). All sample extracts were deidentified prior to inclusion in this study. In total, 3,543 sample extracts were analyzed and processed against an extensive, and continuously updated, in-house library database containing more than 700 drugs, including fentanyl, fentanyl metabolites, fentanyl analogs, other synthetic opioids and drugs of abuse, as well as an extensive number of NPS.

The results from comprehensive data processing included the identification of a wide variety of substances covering all classes and included parent drugs (e.g., fentanyl), metabolites (e.g., norfentanyl), and synthesis precursors (e.g., 4-ANPP) or byproducts (e.g., acetyl fentanyl). For a more accurate determination of drug use, individual identifications were categorized under explicit parent drug groups prior to complex data analysis to determine positivity and combinations. For example, results of fentanyl, norfentanyl, and/or beta-hydroxy fentanyl were all categorized as “fentanyl positive:” 4-ANPP and acetyl fentanyl were not considered for inclusion based on undistinguishable source. Drug classes evaluated included stimulants (e.g., cocaine, methamphetamine, MDMA), opiates/opioids (e.g., heroin, tramadol, buprenorphine), hallucinogens (e.g., ketamine, phencyclidine), and benzodiazepines (e.g., diazepam, alprazolam), as well as these same classes for NPS.

Overall, 1,301 (36.7%) sample extracts were deemed fentanyl positive. The majority (79.8%) of fentanyl positivity was accompanied by poly-drug use, including the presence of one or more drugs of abuse and/or NPS. Fentanyl was found in combination with as many as seven drugs and/or NPS (excluding therapeutics, adulterants, etc.). With respect to drugs of abuse, fentanyl was most commonly found in combination with stimulants (46.0%) and other opiates/opioids (42.8%). Fentanyl was more commonly found in combination with cocaine (26.4%) than methamphetamine (13.1%). Fentanyl combinations with opiates included heroin (28.3%), tramadol (11.1%), and methadone (9.4%). With respect to NPS, fentanyl was most commonly found in combination with synthetic opioids (27.3%) and more rarely found in combination with synthetic stimulants (4.2%), designer benzodiazepines (3.9%), and novel hallucinogens (1.3%).

These results demonstrate the great extent to which fentanyl users are using other substances, either concurrently with or in proximity to their fentanyl use. Fentanyl poly-drug use is significant from forensic toxicology and public health perspectives, as combined drug use creates drug-drug interactions and more complex adverse effect profiles. To better understand poly-drug use, laboratories should consider developing all-inclusive, non-targeted assays for more comprehensive determination of all substances on board at the time of impairment or death.

### Fentanyl, Poly-Drug, Toxicology



## K53 New Trends in Lysergic Acid Diethylamide (LSD) Use and Recommendations for Analysis

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**Learning Overview:** After attending this presentation, attendees will be better informed regarding the prevalence of LSD in postmortem and human performance casework. The expansion of novel LSD analogs will also be discussed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of increasing LSD trends and the importance of having methods in place for sensitive screening and confirmation analyses.

The 1960s were the time of hippies, tie-dye, and LSD. Arguably, this drug defined mainstream recreational drug use of the era with its hallucinogenic effects. While new legal restrictions diminished its popularity in the 1970s, an apparent comeback appears to be on the horizon with more analogs and higher potencies. Over the past five years, the Armed Forces Medical Examiner System–Division of Forensic Toxicology (AFMES-DFT) has observed an upward trend in the detection of LSD in blood and urine specimens. In 2018, 46 cases were confirmed positive for LSD and/or the primary metabolite 2-oxo-3-hydroxy-LSD, as compared to 32 cases from 2015 to 2017. As of July 2019, 34 have already been reported positive, putting this year on track to set a new high if this trend continues.

For screening large batches of casework, it is efficient to use a sensitive LSD immunoassay kit. An immunoassay kit is used at AFMES-DFT that has a Limit Of Detection (LOD) of 0.5ng/mL. Using a 1ng/mL LSD concentration for 100% activity at the decision point, the primary metabolite 2-oxo-3-hydroxy-LSD was calculated to retain only 9% cross-reactivity at the same concentration. This could lead to false negative screens if only the metabolite is present without parent LSD. However, the metabolite is often ten-fold more concentrated than the parent, so the screen may still be successful even with low concentrations of LSD.

With new analogs coming on the market, immunoassays become even more useful to a toxicology lab. LSD analogs all share a similar structure to LSD, with modifications at the N-1 or N-6 positions or substitutions for diethylamide. These analogs can cross-react on an immunoassay and elicit a positive response if at a high enough concentration. Several commercially available reference standards for these analogs were purchased and characterized with the following cross-reactivities: AL-LAD (12%), ETH-LAD (16%), LSZ (18%), 1B-LSD (57%), 1P-LSD (60%), and ALD-52 (83%). This level of cross-reactivity could lead to a number of unconfirmed positive cases if these analogs are not included in the confirmatory testing panel.

A proposed recommendation to prevent this discrepancy is to have a hallucinogenic screening panel targeting LSD, 2-oxo-3-hydroxy-LSD, and their analogs as an escalated testing option when there are clear signs of intoxication without any positive result from routine panels. This can be implemented by using immunoassays, Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), or LC/Time Of Flight/Mass Spectrometry (LC/TOF/MS) analysis. At minimum, one form of analysis should be used on suspected cases.

Case histories may prove especially useful when indicating which cases should proceed to this testing. Examples of indicative histories from casework include those who were running naked through traffic or jumping off buildings with the belief they had the ability to fly. This makes it extremely important for requesting agencies to provide more detailed descriptions about patient behavior in the histories to aid in targeted laboratory testing. It is even more important for labs to test for not only LSD, but also its analogs as their popularity will only continue to increase. Failure to implement appropriate testing for LSD will result in missing confirmations, reporting numerous cases as falsely negative, and underestimating the prevalence of LSD in the community.

### LSD, NPS, Screening



## K54 Xylazine Alone and in Combination With Opioid Drugs in Forensic Toxicology Casework

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**Learning Overview:** After attending this presentation, attendees will understand the frequency of xylazine detection in forensic toxicology casework, the potential effects of the drug alone, and the combined effects with opioids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees about the pharmacology of xylazine and its detection in forensic toxicology casework.

Illicit drugs are often “cut” prior to being sold to the end user. Diluents such as lactose are used to dilute the drug and add bulk to the preparation, but have no pharmacological impact. Adulterants such as procaine may ease the pain of injection and diphenhydramine may enhance the sedative effects of heroin while helping with side effects such as itching. The purpose of this study was to evaluate the frequency of xylazine, a veterinary anesthetic/analgesic, detected alone or in combination with heroin, fentanyl, or fentalogs in blood samples submitted to a large reference laboratory.

Data from cases received between May 1, 2013, and June 30, 2019, and screened by liquid chromatography/time-of-flight/mass spectrometry were extracted from the NMS Labs Laboratory Information Management System. Xylazine was included in the scope of this assay, but routine confirmation and quantification of xylazine did not begin until January 2019. Based on the confirmation rate (approximately 90%) of presumptive positive cases after the availability of the confirmation test, the entire data set was used to evaluate the frequency of xylazine in casework.

One thousand twenty-five cases had a presumptive positive finding for xylazine. Seven cases had no opioids detected and two contained xylazine and methadone. The remaining cases contained one or more of the following opioid drugs: fentanyl (796), heroin (461), morphine (139), 2-furanylfentanyl (26), fluoroisobutyryl/para-fluorobutyrylfentanyl (23), carfentanil (25), 3-methylfentanyl (7), methoxyacetylfentanyl (6), valerylfentanyl (6), cyclopropylfentanyl (5), butyryl/isobutyrylfentanyl (5), U-47700 (3), acrylfentanyl (2), and fluorofentanyl (1). Quantitative data for xylazine were available for 236 cases that had comprehensive screening performed. The mean (range) xylazine concentrations in postmortem ( $N=226$ ) and driving under the influence of drugs ( $N=10$ ) cases were 73 (5–9,100) and 25 (5.3–53) ng/mL, respectively. Fentanyl concentrations were 0.84–420ng/mL in 228 of these cases. Ninety-five cases contained morphine and 40 of these also had 6-Monoacetylmorphine (6-MAM) reported in blood. Mean concentrations for morphine and 6-MAM were 130 and 20ng/mL, respectively. Three postmortem cases that underwent comprehensive screening at NMS Labs and had quantitative confirmation of xylazine contained xylazine in the absence of fentanyl, morphine, or 6-MAM. The information available about these cases is summarized below.

Xylazine (ng/mL)	Other Drugs	History
16	Cis-3-methylfentanyl=0.12ng/mL Bupropion=14ng/mL Hydroxybupropion=360ng/mL Diphenhydramine=150ng/mL Citalopram/Escitalopram=76ng/mL Citalopram/Escitalopram=85ng/mL	Suspected overdose.
9,100		Found in truck with bottle of xylazine and suicide note.
6.6	Metaxlone=0.15µg/mL Citalopram/Escitalopram=390ng/mL	Found having seizures. History of opioid use.

Blood/plasma xylazine concentrations in individuals known to be exposed to xylazine and survived ranged from 570–4,600ng/mL and a postmortem blood xylazine concentration of 16,000ng/mL was reported in a case of intentional intravenous administration. In this study, the xylazine concentrations in cases where opioids were detected are low compared to the reported concentrations in fatal cases, but the potential combined effects cannot be ignored. Reported adverse effects of xylazine include obtundedness, bradycardia, cardiac arrhythmias, and respiratory depression. Therefore, when combined with other central nervous system depressants such as fentanyl or heroin, xylazine may increase risk of overdose in otherwise opioid-tolerant individuals.

### Xylazine, Opioids, Fentalogs



## K55 The Persistence of 3-Methylfentanyl (3-MF) in Pennsylvania

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**Learning Overview:** After attending this presentation, attendees will be able to describe the resurgence of 3-MF in forensic casework between 2016 and 2019, after a nearly 30-year hiatus in Pennsylvania.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by detailing trends regarding the reappearance of 3-MF, including change in positivity over time, demographics, and quantitative data from casework, which will all highlight the unique regional reach of this compound as well as the challenges related to the toxicological confirmation of 3-MF.

In recent years, novel analogs of fentanyl primarily shipped from China have flooded the recreational drug market, but not all emerging substances are newly encountered variants. 3-MF, one of the most potent opioids detected to date, was first encountered in 1979 when it was tied to 100 overdose deaths in California; it was colloquially referred to as “China White.” Two separate incidents of Pennsylvania production were noted in the 1980s. In 1985, the Drug Enforcement Administration (DEA) arrested a chemist for producing and selling 3-MF in Delaware, and in 1988, a second chemist from western Pennsylvania was identified as the source of the drug, which was a factor in 18 fatal overdoses in Allegheny County. Following a nearly 30-year hiatus, in early 2016, 3-MF was reported in seized drug exhibits and drug-related overdose death toxicology tests results from Allegheny and Philadelphia Counties. By 2017, nine total states (Delaware, Maryland, Michigan, New Jersey, New York, Ohio, Pennsylvania, Virginia, and West Virginia) reported 3-MF seizures to NFLIS, with Ohio and Pennsylvania reporting the greatest amounts.

With the reappearance of 3-MF in drug seizures, a toxicological assay was required to confirm its presence in biological specimens. Toxicological analysis of 3-MF has presented significant challenges to toxicology laboratories, due to its potency and presence of its enantiomeric pairs, ( $\pm$ )*cis* and ( $\pm$ )*trans*-3MF. Confirmatory testing has improved over time with ultimately separation and quantitation of *cis* and *trans*-3MF becoming available late 2017 with a reporting limit of 0.05ng/mL using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

Between 2016 and the first half of 2019, 206 blood samples have confirmed positive for the presence of 3-MF. In 2016, 35 cases were reported, which increased to 93 in 2017, dropping to 64 in 2018, and finally to 14 cases during the first half of 2019. These blood samples originated from eight different states (Delaware, Michigan, New Jersey, New York, Ohio, Pennsylvania, Vermont, and West Virginia), with Pennsylvania accounting for 89% of the positives. Within Pennsylvania, 25 different counties reported 3-MF in forensic casework; Montgomery County alone reported 34% of the positives. The toxicology positivity data largely correlates with the geographical distribution of the drug seizure data. Of the 200 cases reporting gender, 70% of the cases involved males while 30% involved females. Age was reported in 184 cases; the average age of an individual involved in a 3-MF case was 35±11 years old, with a reported age range of 1–62 years old.

3-MF has been confirmed in both Driving Under the Influence (DUID) and Postmortem (PM) investigation casework. It has been reported in 29 DUID cases; 10 cases reported 3-MF qualitatively or as a sum of the enantiomers, while 19 cases include isomeric quantitation. Ninety death investigation cases have reported 3-MF isomeric quantitation. A comparison of concentrations for DUID and PM casework is shown in Table 1.

Blood Cases		<i>Cis</i> -3MF (ng/mL)	<i>Trans</i> -3MF (ng/mL)
DUID	N	19	14
	Average ( $\pm$ SD)	0.35±0.24	0.15±0.066
	Range	0.080-1.1	0.05-0.29
PM	N	89	76
	Average( $\pm$ SD)	0.87±1.1	0.36±0.40
	Range	0.060-7.2	0.051-2.2

**Table 1: *Cis* and *trans*-3MF blood concentrations in DUID and PM casework**

3-MF is a highly potent fentanyl analog that is analytically challenging, since it presents itself in two enantiomeric pairs. ( $\pm$ )*Cis*-3-MF is 16 times more potent than fentanyl, and is typically present in higher concentrations than the ( $\pm$ ) *trans*-3MF isomer. It has reappeared in the limited geographical area of Pennsylvania and neighboring states. The limited geographical distribution raises the possibility of local production, reminiscent of the 1980s, as opposed to importation from China.

### 3-Methylfentanyl, NPS, Fentalog



## K56 The Quantitation of *N*-Ethylpentylone (Ephylone) in Blood Samples From Victims of Suspected Drug-Facilitated Sexual Assault

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**Learning Overview:** The goal of this presentation is for attendees to learn about the prevalence and blood concentrations of *N*-ethylpentylone in drug-facilitated sexual assault casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by disseminating information regarding the prevalence of *N*-ethylpentylone, and that it is a concentration in blood specimens from victims of suspected drug-facilitated sexual assaults. This presentation also seeks to describe the associated findings and self-reports from the victims to document voluntary consumption and to ascertain any side effects experienced.

**Background:** The classification of a Drug-Facilitated Crime (DFC) includes Drug-Facilitated Sexual Assaults (DFSA), which can be defined as a sexual activity occurring whereby the victim is incapacitated by drugs and/or alcohol and thereby unable to consent. According to the media, drugs commonly implicated in cases of sexual assault are flunitrazepam (“roofies”), Gamma-Hydroxybutyrate (GHB) and ketamine. Conversely, according to published literature, when examining toxicological results from DFSA victims, ethanol is the most frequently encountered drug, and voluntary drug consumption identified as the highest risk factors for DFSA. While Central Nervous System (CNS) depressants are typically associated with DFSA crimes, any substance that can cause incapacitation through cognitive or psychomotor effects can render someone a victim of non-consensual sexual contact. *N*-1-(1, 3-benzodioxol-5-yl)-2-(ethylamino)-1-pentanone, also known as *N*-ethylpentylone or ephylone, is a synthetic cathinone belonging to the phenethylamine class. *N*-ethylpentylone can cause a state of mental confusion, and when used in combination with other drugs can have a synergistic or additive effect. Side effects from *N*-ethylpentylone use include euphoria, dizziness, vertigo, confusion, tachycardia, and agitation, which is similar to “molly” or “ecstasy,” substances commonly considered to be Methylenedioxymethamphetamine (MDMA), which has been implicated in DFSA cases.

**Method:** All DFSA cases that were submitted to the University of Miami Toxicology Laboratory (UMTL) in 2018 were examined for the presence of *N*-ethylpentylone. Urine specimens were screened and confirmed using a basic liquid-liquid extraction analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Time Of Flight (LC/TOF) instrumentation.

Urine samples were analyzed for ethanol using Headspace/Gas Chromatography/Flame Ionization Detector (HS/GC/FID) instrumentation. *N*-ethylpentylone was confirmed in 11 urine specimens. The associated blood specimens were then sent for quantitation of *N*-ethylpentylone at a reference laboratory (Miami-Dade Medical Examiner, Miami, FL). The case summaries and histories were evaluated in all 11 cases.

**Results:** In 2018, 8% of DFSA cases submitted to the UMTL were positive for *N*-ethylpentylone in the urine specimens. The time from the alleged incident to the time of blood collection ranged from 5 to 56 hours. Five of the 11 cases confirmed *N*-ethylpentylone in the blood specimens at less than the lower limit of quantitation (10ng/mL). Two cases had no detectable *N*-ethylpentylone in the blood specimen. Of the 4 cases where quantitative values were within the linear range, the concentrations ranged from 10–46ng/mL. None of the 11 cases had detectable alcohol in the urine or blood specimens, and 7 cases had other drugs present, some of which were reported as ingested by the subjects. Other drugs identified include benzodiazepines (alprazolam and clonazepam), cocaine, antidepressants (citalopram, quetiapine, and fluoxetine), and cannabis.

Information obtained from the toxicology questionnaire for sexual assault cases documented that 7 out of the 11 victims reported using cannabis recently. One of the victims reported using recreational “molly,” another reported that she had been forced to ingest “ecstasy” before the incident occurred. Common side effects reported include: drowsiness (73%), sedation (73%), confusion (45%), memory loss (45%), blackout (45%), and hallucinations (27%).

**Conclusion:** Despite the literature documenting the prevalence of alcohol in DFSA cases, alcohol was not detected in the blood or urine specimens in any of the cases in which *N*-ethylpentylone was identified. From the results presented, testing for designer drugs, including the synthetic cathinones, should be incorporated into routine DFSA casework, even if not indicated by the victim. The side-effect profile of *N*-ethylpentylone could lead a victim to be vulnerable and more susceptible as a victim of non-consensual sexual contact.

### *N*-Ethylpentylone, Sexual Assault, Side Effects



## K57 Buprenorphine-Related Deaths in North Carolina From 2010 to 2018

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**Learning Overview:** After attending this presentation, attendees will understand the epidemiology and the increase in buprenorphine deaths in North Carolina and will gain an appreciation of the pharmacology of buprenorphine in combination with other Central Nervous System Depressant (CNSD) substances, including therapeutic prescription medication. In addition, attendees will understand how analytic, autopsy, and investigation information may be critical to determine cause of death in challenging buprenorphine toxicity cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of the complex mechanism of action of buprenorphine and norbuprenorphine. The additive effects of CNSDs, especially those drugs that interact with P-glycoprotein (P-gp), will be discussed. This will improve the understanding and interpretation of postmortem buprenorphine cases and emphasize the need for correlation with death investigation information.

Between 2010 and 2018, there were more than 100 cases in which buprenorphine was detected in the peripheral blood and considered the cause of death with *no other opioids* or substances found in the supratherapeutic range. Of these cases, 65% were male. The ages of decedents varied widely, with a range of 14–63 years. The mean/median peripheral blood concentrations for parent and metabolite were 4.5/2.3ng/mL and 8.2/3.5ng/mL, respectively. These concentrations overlap those reported in the literature for opioid-dependent subjects receiving sublingual maintenance therapy. The route of administration, if available, scene findings, and decedent behavior prior to death were strongly considered by the pathologist for each case to provide context to the drug cause of death. For these data, a query was performed in the North Carolina Controlled Substances Reporting System for each decedent. For this particular set of buprenorphine cases, the decedents would have presumably survived the ingestion of other CNSDs, or therapeutic medications, if not for the presence of buprenorphine and its metabolite.

A majority of the buprenorphine deaths involve decedents with underlying cardiovascular disease. This raises the possibility that individuals with underlying cardiovascular disease may be more susceptible to the toxic effects of buprenorphine. While safer than other opioid alternatives, buprenorphine may not be void of effects on the heart, especially when not used as prescribed, and thus predispose individuals with heart conditions to an untimely death.

Determining cause of death in buprenorphine-related cases can be difficult. Deaths from buprenorphine may be underestimated in epidemiological data because of the lack of a defined toxic or lethal range involving a drug with a good safety profile, especially when compared to other opioids used for medication-assisted treatment. As the treatment of opioid-use disorder becomes a priority, more awareness of the challenges of postmortem interpretation is needed in the forensic community as increased use and diversion of buprenorphine are inevitable.

### **Buprenorphine, Postmortem, Opioid**



## K58 Evaluation of the Long-Term Stability of Select Phenylacetylindole, Cycloalkylindole, Quinoliny, and Carboxamide Synthetic Cannabinoids in Human Whole Blood Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Overview:** After attending this presentation, attendees will learn to assess the stability of synthetic cannabinoids in human whole blood using LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing long-term stability results of synthetic cannabinoids under various storage conditions.

**Background/Introduction:** Despite efforts to control synthetic cannabinoids, clandestine manufacturers continue to modify their structures to avoid legal consequences, creating an ever-changing analytical target for forensic laboratories. Forensic toxicology laboratories often lack the needed resources or do not have the capabilities to test for these compounds and metabolites, requiring specimens to be submitted to reference laboratories. Drug stability can be affected by long storage times, temperature, and preservatives. Although these factors can be controlled, systematic research is necessary to identify their impacts on the stability of these new synthetic cannabinoids that are continually emerging.

**Objective:** The purpose of this research was to assess the stability of 17 synthetic cannabinoids in human whole blood using LC/MS/MS over a 35-week period. The method was validated in accordance to the Academy Standards Board method validation guidelines for quantitative analysis and stability evaluation of the following analytes: phenylacetylindoles JWH-250 and RCS-8; cycloalkylindoles UR144 and XLR11; quinoliny PB-22 and NM2201; and carboxamides 4-cyano CUMYL-BUTINACA, 5-fluoro-3,5-ABPFUPPYCA, 5-fluoro ADB-PINACA, 5-fluoro PY-PINACA, ADB-PINACA, APP-PICA, CUMYL-THPINACA, EMB-FUBINACA, MDMB-FUBICA, MEP-CHMICA, and MO-CHMINACA.

**Methods:** Stability under room temperature (20°C), refrigerator temperature (4°C), and freezer temperature (-20°C) at high (10ng/mL) and low (1.5ng/mL) concentrations each in triplicate were evaluated at ten selected time points: 0-hour, 24 hours, 72 hours, 1 week, 3 weeks, 5 weeks, 9 weeks, 17 weeks, 21 weeks, and 35 weeks. Blood was preserved with sodium fluoride prior to the stability study. Extraction of analytes was conducted using Supported Liquid Extraction (SLE+) ISOLUTE® cartridges. The extracts were analyzed using a Waters® Xbridge™ reverse-phase C18 column (3.5µM, 2.1 x 50mm) by Shimadzu® HPLC with a SCIEX™ 4000 Q-Trap Electropray Ionization/Tandem Mass Spectrometry (ESI+/MS/MS) in positive ionization mode. The total run time was eight minutes with a 0.6mL/min flow rate and 10µL injection volume.

**Results:** Linear calibration curves for each analyte had acceptable R<sup>2</sup> values >0.99 using a weighting factor of 1/x. A linear dynamic range of 0.5ng/mL to 25ng/mL was used for all analytes within acceptable ±20% calculated bias and imprecision, except NM2201 and APP-PICA had a Limit Of Quantitation (LOQ) of 0.1ng/mL and MO-CHINACA had a working range of 0.5–15ng/mL. No signs of carryover were observed. Analytes were considered stable if the average area ratio at the time point examined was within ±20% of the average area ratio response at time point zero. Phenylacetylindole, cycloalkylindole, and carboxamide analytes were stable up to 35 weeks under all temperatures, such as JWH-250, 5-fluoro PY-PINACA, and UR144, with core structures of a carbonyl substituent on a pyrazole or pyrrole with surrounding non-polar groups of hydrocarbons and heterocyclic rings. Compounds with two polar carbonyl functional groups present, such as EMB-FUBINACA, 5-fluoro ADB-PINACA, APP-PICA, and MEP-CHMICA, were found to experience degradation earlier at one week or less in room temperature and refrigerated storage conditions. 5-fluoropentyl analogs, such as XLR11 and 5-fluoro ADB-PINACA, in comparison to their counterpart analytes, UR144 and ADB-PINACA, were unstable at earlier time points of less than one week under room temperature and/or refrigeration.

**Conclusion:** The validated method demonstrates a sensitive and reliable way to positively identify 17 different synthetic cannabinoids in human whole blood in rapid time for stability analysis. Further, the use of SLE improved sample preparation efficiency by decreasing the extraction time from 1 hour to 30 minutes compared to traditional extraction methods, such as solid-phase extraction and liquid-liquid extraction. The select 17 synthetic cannabinoids generally degraded in the order of room temperature, refrigerator, then freezer temperature. Long-term stability results revealed that the overwhelming majority of synthetic cannabinoids were stable up to 35 weeks when kept frozen.

### Synthetic Cannabinoids, Supported Liquid Extraction, Stability



## K59 The Quantitative Analysis of Fentanyl and Fentanyl Analogs in Hair

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**Learning Overview:** After attending this presentation, attendees will know how to detect fentanyl use through hair analysis. Attendees will also know how to identify the presence of fentanyl analogs and their metabolites in hair.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a method for the detection and quantitation of fentanyl, fentanyl analogs, and their metabolites in hair. Data on the prevalence of fentanyl analogs that are also appearing in fentanyl users' hair will also be presented.

**Hypothesis:** Fentanyl, fentanyl analogues, and their metabolites are detectable in the hair of fentanyl users. The fentanyl can be extracted and analyzed via microwave extraction, solid phase preparation, and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis.

An LC/MS/MS method for the analysis of fentanyl and fentanyl analogues in hair was developed. The assay was validated for fentanyl, norfentanyl, acetyl fentanyl, acetyl norfentanyl, furanyl fentanyl, furanyl norfentanyl, carfentanil, norcarfentanil, butyryl fentanyl, butyryl norfentanyl, valeryl fentanyl, and para-fluoro-butyryl fentanyl. The method was validated for precision, accuracy, linearity, carryover, interference, matrix suppression, recovery, and dilution integrity.

The fentanyl was extracted from the hair via microwave extraction in 9:1 methanol:trifluoroacetic acid. The solid phase clean-up was performed with DPX-CX tips on a Hamilton® STARlet liquid handler. The samples were sequentially washed with 0.1M acetate buffer pH 4.0, methanol/0.1 M HCl (4:6), and methanol, and eluted with 80:20:3 methylene chloride:isopropanol:ammonium hydroxide. The eluent was dried and reconstituted in mobile phase. The analysis was performed on a QTRAP® 6500+ LC/MS/MS with a Shimadzu® LC30-AD binary pump system and PAL-HTCxt with a DLW wash system. A Phenomenex® Kinetex® C18 150 x 2.1 mm, 1.7 µm particle size, 100 Å pore size Ultra Performance Liquid Chromatography (UPLC) column using a gradient elution with 0.1% formic acid in water and 0.1% formic acid in methanol was employed for chromatographic separation.

The presence of fentanyl was confirmed in 110 samples that had previously been screened by enzyme immunoassay for fentanyl. The sample pool utilized for the screening consisted predominantly of samples that had previously identified as positive for heroin metabolite (6-monoacetylmorphine, morphine). Many of the samples were also positive for other street drugs, mostly cocaine and methamphetamine.

The median concentration for the fentanyl was 132.5pg/mg. The median concentration for norfentanyl was 10.4pg/mg. The average ratio of fentanyl to norfentanyl was 20:1.

Many of the 110 positive samples contained other fentanyl derivatives. Furanyl fentanyl was detected in 55% of the samples; and butyryl fentanyl, carfentanil, and acetyl fentanyl were detected in a smaller fraction of the samples. No valeryl fentanyl or para-fluoro butyryl fentanyl was detected in any of these samples. The normetabolites of the fentanyl derivatives were also detected in samples with high concentrations (typically >100pg/mg) of the parent drug.

No samples were identified that had fentanyl analogs to the exclusion of fentanyl. This is somewhat due to poor cross-reactivity of some of the fentanyl analogs in the immune-assay, although butyryl fentanyl, valeryl fentanyl, furanyl fentanyl, and acetyl fentanyl have 100% or better cross-reactivity.

This method was used to successfully extract and analyze fentanyl, fentanyl analogs, and their metabolites in hair. Samples were identified containing fentanyl, furanyl fentanyl, carfentanil, butyryl fentanyl, and acetyl fentanyl.

### Fentanyl, Analogs, Hair



## K60 Physicochemical Characterization of 19 Fentalogs: Lipophilicity

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**Learning Overview:** After attending this presentation, attendees will have increased understanding regarding the physicochemical properties of fentanyl analogs (fentalogs) and their importance in forensic science.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing additional information concerning the lipophilicity of fentalogs and their overall impact in terms of analytical separations and the interpretation of results.

Forensic toxicologists are tasked with the identification of New Psychoactive Substances (NPS) in biological matrices and may be required to interpret their findings. Yet, the physicochemical properties of these new and emerging drugs are not always known. Although fentanyl and a small number of derivatives used for medical or veterinary procedures are well characterized, physicochemical properties have not been determined for many emerging fentanyl analogs. Lipophilicity plays an important role in terms of separation and analytical determination, as well as the overall pharmacology of the drug (i.e., absorption, distribution, metabolism, and excretion). It is described by parameters such as partition coefficients ( $\log P/\log D$ ) and Volume of distribution ( $V_d$ ).

Partition coefficients were determined for 19 fentalogs. Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) was used to experimentally determine partition coefficients (octanol/water) for (+)-cis-3-methylfentanyl, 4-ANPP, acetylfentanyl, butyrylfentanyl, isobutyrylfentanyl, furanylfentanyl, valerylfentanyl, norfentanyl, norcarfentanyl, alfentanil, remifentanil, sufentanil, carfentanil, p-fluoroisobutyrylfentanyl, p-fluorobutyrylfentanyl, p-fluorofentanyl, o-fluorofentanyl, and  $\beta$ -hydroxythiofentanyl. An Agilent® 1290 Infinity LC coupled to an Agilent® 6470 triple quadrupole mass spectrometer was employed in positive Electrospray Ionization (ESI) mode. Fentalogs were separated using gradient elution using a mobile phase consisting of deionized water/acetonitrile containing 0.1% formic acid. Data were acquired using Multiple Reaction Monitoring (MRM) and data analysis was performed using Agilent® MassHunter™ software. Source conditions were optimized for all analytes and chromatographic resolution was achieved for all 19 fentalogs.

Experimentally determined partition coefficients were compared with specialized software-generated data from three independent sources (ACD, ALogPS, and LogKOW).  $\log D$  values for the 19 fentalogs were measured over a wide range (<0.1 to 2.7) in octanol/water. Partition coefficients were also evaluated over a range of pH values in aqueous buffer systems. The results highlight important differences in the lipophilicity of fentalogs with phenethyl, piperidine, aniline, or *N*-propionyl substitutions. Although numerically determined partition coefficients can be of value for forensic chemistry and toxicology applications, the comparison of experimental and software-generated data can provide important insight regarding future fentalogs that have yet to emerge. Per research, this study may be the largest characterization of fentanyl lipophilicity to date. The observed differences in lipophilicity can have important implications in terms of the pharmacology and toxicology of these synthetic opioids.

### Fentalogs, Partition Coefficients, $\log P/\log D$



## K61 Identification of Mitragyna Alkaloids and Metabolites as Biomarkers of Kratom Use in Postmortem Urine Samples

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**Learning Overview:** After attending this presentation, attendees will be familiar with the identification of mitragyna alkaloids in biological samples using Liquid Chromatography/quadrupole/Time Of Flight/Mass Spectrometry (LC/qTOF/MS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying additional alkaloids of *Mitragyna speciosa* as biomarkers of kratom use. In addition, the merits of enzymatic and chemical hydrolysis of phase II metabolites will be discussed.

*Mitragyna speciosa* (kratom) is a tree native to Southeast Asia that has been utilized as both a medicine and cultural tradition by the local populace. The plant is known to have dose-dependent effects—in low doses it produces a stimulant effect, and in high doses an opiate effect. Although not federally regulated, kratom's unique effects and unregulated status make it an ideal target for recreational drug use. The plant is known to contain more than 20 corynanthe-type indole alkaloids, of which the primary psychoactive components are Mitragynine (MG) and 7-Hydroxymitragynine (MG-OH). Other prominent compounds of kratom include two diastereoisomers of mitragynine (Speciogynine (SG) and Speciociliatine (SC)) and another structurally similar alkaloid, Paynantheine (PY). These three alkaloids are not reported to be psychoactive. In addition, MG's metabolism has been studied and several prominent phase I metabolites have been identified including 16-Carboxymitragynine (16-COOH-MG), 9-O-Demethylmitragynine (9-O-DM-MG), and most notably, MG-OH. Following the administration of kratom, biological samples may contain a mixture of these structurally similar mitragyna alkaloids in addition to their metabolites. This can present a significant challenge in terms of analytical detection.

A previously validated LC/qTOF/MS assay was used for the quantitative and qualitative identification of four kratom alkaloids (MG, MG-OH, SG, and SC) in postmortem urine. A modified LC/qTOF/MS assay was also used to further investigate phase I and phase II metabolites of MG among these kratom users. Chemical and enzymatic hydrolysis of glucuronides and sulfates were evaluated. In the absence of reference standards, phase I metabolites were identified using high-resolution mass spectra and retention time matching. Recombinant cytochrome P450 enzymes were used to generate phase I metabolites *in vitro* for this purpose.

Using unhydrolyzed specimens, parent drug (MG) was identified in all samples tested over a concentration range of 26–1,987ng/mL. Other kratom alkaloids were also detectable in all 16 samples with concentrations ranging from 2–317ng/mL (PY), 59–1,684ng/mL (SG), and 23–3,309ng/mL (SC). Notably, among 12 of the 16 samples, concentrations of speciociliatine, speciogynine (or both alkaloids) exceeded that of MG. MG-OH, which is known to be unstable, was detected in nine samples. 9-O-DM-MG was identified in 12 of the 16 samples, but 16-COOH-MG was identified in only one specimen.

Deconjugation using recombinant enzymes proved more effective than either chemical hydrolysis or enzymatic hydrolysis using traditional glucuronidase and sulfatase preparations. Detectability of the carboxylated metabolite (16-COOH-MG) significantly increased following hydrolysis. However, the abundance of 9-O-DM-MG and MG-OH were only marginally improved by the additional sample preparation step, and stability issues were identified. Overall, deconjugation of phase II metabolites in urine proved to be of little benefit.

Given the increase of kratom usage in the United States and its reported use for the non-medically supervised treatment of opioid abstinence syndrome, identification strategies are needed. In addition to MG and MG-OH, several other alkaloids (SC, PY, SG) can be used to identify kratom use. In addition to these potential biomarkers, 9-O-DM-MG was the most abundant metabolite and was readily detected without deconjugation. However, until an analytical reference standard is available, it is unlikely to be incorporated into routine testing.

### Kratom, Urine, Biomarkers

## K62 Rapid Detection and Separation of Isomeric Fentanyl Analogs Using Gas Chromatography-Atmospheric Pressure Chemical Ionization-Trapped Ion Mobility-Time of Flight/Mass Spectrometry (GC-APCI-TIMS-TOF/MS)

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**Learning Overview:** After attending this presentation, attendees will better understand a novel instrumentation technique for the separation and identification of isomeric fentanyl analogs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing an alternative high-resolution instrumentation technique for toxicological analysis that is complementary to traditional chromatography/mass spectrometry workflows.

The emergence of Novel Psychoactive Substances (NPS) in the United States, especially fentanyl analogs, has led to the need for alternative screening and quantitative techniques capable of achieving both high sensitivity and specificity. Due to the clandestine nature of these compounds, the potential for the development of isomeric sets is high, thus increasing the need for method development of a complementary separation technique for the detection and quantification of isomeric substances. With relatively unknown potencies among structural isomeric and isobaric sets, being able to distinguish between these compounds is paramount when properly certifying the cause and manner of death. In the present work, the use of complementary gas-phase separation was evaluated for the analysis of fentanyl analogs, specifically GC-APCI coupled to TIMS-TOF/MS.

Sixteen fentanyl analogs, as shown in Table 1, including six sets of isomers, were prepared in methanol using certified reference standards provided by the Miami-Dade Medical Examiner Department (MDME). All standards were fortified into whole blood and extracted via protein precipitation with acetonitrile. In addition, previously analyzed postmortem case samples, provided by the Institute of Forensic Medicine, Medical Center at the University of Freiburg, Germany, were also extracted and sampled to confirm the application of the method developed. Chromatographic separation was achieved using a GC equipped with a DB-5 non-polar capillary column, coupled to an APCI source in positive ionization mode. A 15min thermal ramping method was used, with compound elution between 6-10 minutes of the total run time. Using the standards provided, the GC-TIMS-MS method was optimized, and detection of analogs was achieved using a commercial TIMS-TOF with an internal mobility and accurate mass calibration.

**Table 1. Analytes**

Despropionyl ortho-fluoro fentanyl (298 Da)	Cyclopropyl fentanyl (348 Da)	Benzyl fentanyl (322 Da)	3-methyl fentanyl (350 Da)	Para-fluorobutyryl fentanyl (368 Da)	Meta-fluoro fentanyl (354 Da)	Fentanyl (336 Da)
Despropionyl para-fluoro fentanyl (298 Da)	Crotonyl fentanyl (348 Da)	Acetyl fentanyl (322 Da)	Butyryl fentanyl (350 Da)	Para-fluoroisobutyryl fentanyl (368 Da)	Ortho-fluoro fentanyl (354 Da)	Furanyl fentanyl (374 Da)
					Para-fluoro fentanyl (354 Da)	Methoxyacetyl fentanyl (352 Da)

All fentanyl analogs were positively identified using high resolution mass accuracy, with experimental  $m/z$  values within 1ppm of calculated theoretical  $m/z$  value. All analytes were linear from 1ng/mL to 500ng/mL post-extraction and no endogenous or exogenous interferences were observed. An increase in signal to noise was noted when signals were filtered in the GC, IM, and MS domains, allowing for increased sensitivity. A Collision Cross Section (CCS) value, which is a term that represents the interaction between the target ion and the drift gas ( $N_2$ ) in the TIMS cell, is used to characterize the measured IM of a compound. CCS was measured for all fentanyl analogs (<1% error) and mobility separation was classified as a difference in CCS value larger than  $0.6 \text{ \AA}^2$ ; this separation was achieved for four out of the six sets of structural isomers. All analytes were also observed as having a characteristically high mobility resolving power ( $R > 100$ ). In addition, five out of the six sets of isomers were chromatographically resolved ( $r > 1$ ). Successful separation was achieved for each set of isomers either by IM, chromatography, or both. The analytes detected in the previously analyzed postmortem case samples, including furanyl fentanyl, and methoxyacetyl fentanyl, were confirmed positive on the present method, with quantitative values within 20% of the previously measured concentrations. The use of GC-IM spectrometry for the detection of fentanyl analogs in the field of toxicology demonstrates the advantage of orthogonal separation techniques and multi-dimensional identification (retention time, CCS value, and accurate mass). Ongoing experiments will assess the use of liquid chromatography, as well as the addition of more analytes to the method.

### High Resolution Mass Spectrometry, Novel Psychoactive Substances, Ion Mobility

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## K63 Determination of Novel and Non-Routine Benzodiazepines and Suvorexant in Whole Blood by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Overview:** After attending this presentation, attendees will understand the benefits and limitations of an efficient analytical method for the determination of select benzodiazepines and suvorexant in whole blood by LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a validated method for the detection of select benzodiazepines and suvorexant in whole blood.

**Introduction:** Benzodiazepines are frequently encountered in human performance forensic toxicology cases, such as drug-facilitated assaults and impaired driving, due to their effect on the central nervous system. The growing trend of Novel Psychoactive Substance (NPS) -benzodiazepines has increased the need to expand the scope of targeted assays. In the past 19 months, the Palm Beach County Sheriff's Office Crime Lab has received drug seizure submissions containing clonazepam, etizolam, flualprazolam, and flubromazolam.

**Objective:** Develop and validate an efficient method for quantitative determination and/or qualitative identification of select novel and non-routine benzodiazepines and suvorexant in whole blood by LC/MS/MS.

**Method:** Two hundred microliters of whole blood was prepared by protein precipitation using LC/MS-grade acetonitrile. Analysis of the samples was performed on a Shimadzu® Prominence XR LC system with a SCIEX® 3200 QTRAP® MS/MS and a Raptor™ Biphenyl column with dimensions of 50 x 2.1mm and a 2.7µm particle size. A gradient LC program with a flow rate of 0.6mL/minute was used with LC/MS-grade water and acetonitrile, both with 0.1% formic acid. The total run time was eight minutes. Stable isotopically labeled internal standards of bromazepam, clonazepam, clobazepam, diclazepam, estazolam, etizolam, suvorexant, and triazolam were used. The method was validated by evaluating the calibration model, sensitivity, carryover, selectivity/specificity, repeatability, bias, robustness, a case sample evaluation, ionization suppression/ enhancement, and extract stability.

**Results:** Two different quantitative ranges were employed based on anticipated concentrations of each analyte in whole blood. The range for Group 1 (clonazepam, diclazepam, flualprazolam, flubromazepam, flubromazolam, loprazepam, lormetazepam, and triazolam) was 5–100ng/mL. The range for Group 2 (bromazepam, clobazepam, clonazepam, estazolam, etizolam, phenazepam, prazepam, suvorexant, and tetrazepam) was 25–500ng/mL. Flubromazepam and prazepam were validated for qualitative identification only. The Limit Of Detection (LOD) was 1ng/mL for Group 1 (except clonazepam, flubromazolam, and triazolam, which was 5ng/mL) and 5ng/mL for Group 2. The Limit Of Quantitation (LOQ) was administratively set to the lowest calibrator. The calibration model for all analytes used a quadratic curve fit with 1/x weighting. Within-run ( $n=3$ ) and between-run ( $n=15$ ) precision for the quantitative analytes did not exceed 14% for controls prepared at the LOQ (in five different matrix sources), a midpoint concentration, and a concentration within 20% of the highest calibrator. Bias did not exceed  $\pm 14\%$ . An evaluation with previously characterized case samples ( $n=19$ ) demonstrated interference for one of the ion transitions for triazolam in one case that may interfere with its identification. There was no interference with other common analytes from drug classes, such as stimulants, tetrahydrocannabinols, opioids, and other benzodiazepines. Ionization suppression/enhancement of the target response relative to the internal standard was not greater than  $\pm 25\%$  for all analytes, except prazepam at the LOQ and loprazepam at both the LOQ and a concentration within 20% of the highest calibrator. Extract stability of flubromazepam did not exceed 24 hours (peak area varied by  $\geq 20\%$ ). Tetrazepam and triazolam demonstrated stability up to 48 hours. Clonazepam, lormetazepam, and phenazepam demonstrated stability up to 72 hours. All other compounds demonstrated stability up to 96 hours. A certified reference material for suvorexant was not available and therefore will be reported qualitatively even though all validation requirements were met. Flubromazepam and prazepam demonstrated bias  $> \pm 20\%$  with control replicates prepared in alternate matrix sources at the LOQ. Stable isotope-labeled internal standards may be required for quantitation of those analytes by this method.

**Conclusion:** The presented method requires a small volume of sample, minimal sample preparation, and demonstrated acceptable performance for the quantitative analysis of 15 of the 17 compounds evaluated. This method allows for the quantitative determination and/or the qualitative identification of 16 novel and non-routine benzodiazepines and suvorexant in whole blood by LC/MS/MS.

### Benzodiazepines, Validation, LC/MS/MS



### **K64 Fentanyl Epidemic on the West Coast: Accidental Overdose Death Trends in San Francisco From 2008 to 2019**

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**Learning Overview:** After attending this presentation, attendees will understand how fentanyl has become an alarming epidemic on the West Coast, as well as what the trends within the drug use and deaths are. Additionally, this presentation will highlight how the fentanyl epidemic impacts local communities, medical examiner offices, and public health as a whole.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering insight to trends among sole fentanyl consumption, as well as co-consumption with other drugs, such as medicinal opioids, heroin, methamphetamine, and cocaine. These trends may shed light onto how agencies can better address the epidemic in local communities, including the implementation of preventative strategies. Importantly, this data highlights how forensic laboratories can better observe the issue through an efficient and systematic toxicological approach. Furthermore, this presentation may highlight the need for more uniformity across and within forensic labs in terms of medical death definitions (both cause and manner of death classifications) and methods and regime of toxicological analyses.

Fentanyl is most widely known for its medical uses, but there has been a trend, both locally and nationwide, that illicit drugs are being laced with fentanyl, and agencies are more often detecting fentanyl with other illicit drugs. The addition of fentanyl and fentanyl analogs into, or consumed with, other drug products can produce potentially dangerous consequences, even to opioid-tolerant individuals who may be unaware of such additions.

It was the goal of this research to uncover the trend of fentanyl-related deaths in the City and County of San Francisco from 2008 to 2019. Since 2014, there has been a sharp increase in fatal overdoses involving fentanyl in San Francisco, which surpassed fatal overdoses involving heroin and/or opioids by 2016. In 2015, 2016, 2017, and 2018, there were approximately 18, 23, 43, and 104 deaths involving fentanyl, respectively, with almost all of those deaths being classified as accidental overdoses.

Opioid-like overdose symptoms in stimulant users led to the discovery of fentanyl-laced cocaine and methamphetamine, where many users were hospitalized and/or died as a consequence. From 2016 onward, the presence of fentanyl in methamphetamine- and cocaine-related deaths has also increased significantly.

Outbreaks of fentanyl analogs have also been observed since late 2015 when the ingestion of furanyl fentanyl by a young male in 2017 led to his death. Fentanyl analog inclusion was typically seen in counterfeit medication or recreational pills. Like methamphetamine and cocaine users, victims seemingly had no indication of previous fentanyl or fentanyl analog use, highlighting that tolerance to such potent fentanyl and fentanyl analogs plays a factor in opioid-naïve users.

The results have shown that there indeed is an upward trend of the detection of fentanyl in death cases, both in accidental overdoses and non-accidental overdoses. These trends must first be detected, then considered, and continually captured for the interests of law enforcement agencies, forensic laboratories, and, importantly, given to public health agencies for drug harm reeducation measures.

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#### **Fentanyl Epidemic, Forensic Toxicology, Opioids**



## K65 Accidental Acute Combined Drug Toxicity Involving Heroin and Fentanyl in a 10-Year-Old Child

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**Learning Overview:** The goal of this presentation is to describe the circumstances and postmortem toxicological findings of a case involving an accidental acute heroin and fentanyl overdose in a 10-year-old child.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of the severity of the national opioid epidemic.

A 10-year-old Black male was found unresponsive at home by his mother, lying facedown on a couch in cardiac arrest. Emergency medical services responded to the scene and initiated Cardiopulmonary Resuscitation (CPR). The decedent exhibited trismus, resulting in the administration of 10mg of midazolam in order to secure an airway. He was transported via ambulance to the emergency room but was pronounced dead shortly after arrival. Naloxone was never administered, most likely due to the decedent's age and lack of circumstantial evidence at the scene to suggest opioid abuse. According to the decedent's uncle, he had spent the day swimming with his family at the community pool and was observed awake and alert an hour before being discovered unresponsive by his mother. He had eaten a snack, vomited, and sat down on the couch to watch television.

At autopsy, the medical examiner noted that the decedent had aspirated his gastric contents and exhibited pulmonary congestion and cerebral edema with no other remarkable findings. Central and peripheral blood, vitreous humor, and total gastric content were collected and submitted for toxicological analysis. Comprehensive toxicology testing was performed, including a volatiles screen, a 5-assay Enzyme-Linked Immuno-Sorbent Assay (ELISA) screen, and a blood drug screen by both Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Ion Trap/Mass Spectrometry (LC/Ion Trap/MS<sup>n</sup>). Morphine, 6-acetylmorphine, codeine, fentanyl, and midazolam were identified in the decedent's peripheral blood and were quantified by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). 6-acetylmorphine and fentanyl were confirmed in the decedent's vitreous humor and were also quantified in the decedent's gastric content. Postmortem concentrations of detected analytes were as follows:

**Table 1:** Postmortem Concentrations of Detected Analytes

Analyte	Specimen	Concentration
Morphine	Blood – Iliac Vein	0.015mg/L
6-Acetylmorphine	Blood – Iliac Vein	<1.0ng/mL
	Gastric Content	<1.0ng/mL
Codeine	Blood – Iliac Vein	<0.010mg/L
Fentanyl	Blood – Iliac Vein	15.5ng/mL
	Gastric Content	6.5ng total
Midazolam	Blood – Iliac Vein	0.100mg/L

Based upon the autopsy results and the toxicology findings, the medical examiner determined the cause of death to be an acute combined drug toxicity involving heroin and fentanyl. The manner of death was ruled an accident. Although the media speculated that the decedent found the drugs either at the pool facility, on his way home, or in his home, video footage was obtained showing the decedent acting normally while walking to his house from the pool facility, and law enforcement concluded that there was no reason to believe the drugs were in the house. Despite the extensive investigation, little to no additional information was revealed regarding how and where the decedent came in contact with heroin and fentanyl. This case demonstrates the reach of the national opioid epidemic and that victims can come from all different demographics.

### Fentanyl, Heroin, Postmortem



## K66 A Fatal Mono-Intoxication With 4-Fluoroisobutyrylfentanyl

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**Learning Overview:** After attending this presentation, attendees will understand the need to share and publish postmortem toxicological data on new psychoactive substances such as fentanyl analogs for toxicological interpretation in new casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing toxicological data on the presence and concentration of 4-fluoroisobutyrylfentanyl, a new psychoactive substance, in various biological samples.

During the past decades, there has been an increase in the appearance of fentanyl analogs as new psychoactive substances. Fentanyl is a synthetic narcotic opiate with strong analgesic properties and is about 100 times more potent than morphine. Because of its high potency, fentanyl and fentanyl derivatives are misused and abused and may cause death. Limited toxicological data are available on these new fentanyl derivatives. In this presentation, new toxicological data of a fentanyl derivative in a postmortem case are presented. The goal was to explain the cause of death of a 35-year-old man who was encountered in need of resuscitation in his bathroom. He died shortly afterward. Drug paraphernalia (including a base pipe) and a small amount of rough material were found near the victim.

At the Drugs Department, the rough material was investigated using Gas Chromatography/Mass Spectrometry (GC/MS), Infrared (IR) spectroscopy, and Nuclear Magnetic Resonance (NMR). At the Toxicology Department, the body material obtained from autopsy was investigated for toxic substances following the standard screening protocol on ethanol, drugs, medicines, and pesticides using Headspace/Gas Chromatography/Flame Ionization Detection (HS/GC/FID), Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS), GC/MS, and Ultra-Performance Liquid Chromatography/Diode-Array Detection (UPLC/DAD). Furthermore, 4-Fluoroisobutyrylfentanyl (4-FIBF) was subsequently identified and quantified using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

Toxicological analysis on the body material showed the presence of 4-FIBF. With the chromatographic method, the separation of the 2, 3, and 4-fluorobutyrylfentanyl and 2, 3, and 4-fluoroisobutyrylfentanyl isomers could not be confirmed. However, the fentanyl derivative was identified in the rough material as almost pure 4-FIBF. Therefore, it is very likely that the derivative found in the body material was 4-FIBF. Concentrations of 4-FIBF were measured in various biological samples, including femoral blood, heart blood, vitreous humor, brain tissue, liver tissue, and urine. The measured concentrations were respectively 0.030mg/L; 0.12mg/L; 0.067mg/L; more than 0.081mg/kg; 0.44mg/kg, and between 0.0010 and 0.025mg/L. No other compounds were found.

In literature, only two cases were found with toxicological data on 4-FIBF. In a publication by M. Rojkiewicz et al. in 2017, the concentrations of 4-FIBF measured were higher compared to this case although it was not clear whether the analyzed blood was peripheral blood or heart blood.<sup>1</sup> Based on the toxicological results, it was concluded that the measured concentrations of 4-FIBF could have contributed to or possibly explain the cause of death if no other plausible cause was found. The additional role of heart muscle inflammation seen at autopsy could not be completely excluded.

In conclusion, this case showed a fatal intoxication of 4-FIBF. The presence and concentration of 4-FIBF was measured in various biological samples. Because only two other cases with data on 4-FIBF were published, these additional postmortem toxicological data on this new psychoactive substance are very useful for the toxicological interpretation.

### Reference(s):

1. M. Rojkiewicz, M. Majchrzak, R. Celiński, P. Kuś, M. Sajewicz. Identification and physicochemical characterization of 4-fluorobutyrylfentanyl (1-((4-fluorophenyl)(1-phenethylpiperidin-4-yl)amino)butan-1-one, 4-FBF) in seized materials and the postmortem biological samples. *Drug Test. Anal.* 2017, 9, 405.

### 4-Fluoroisobutyrylfentanyl, Fatal Intoxication, Postmortem

## K67 Circumstances, Postmortem Findings, and Toxicology in a Series of Methoxyacetylfentanyl-Related Deaths

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**Learning Overview:** The goal of this presentation is to increase the knowledge of the toxicology of Methoxyacetylfentanyl (MAF) by providing quantitative data in postmortem cases and discuss cases of fatal intoxications.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the awareness of new non-routine drugs, the toxicology of MAF, and provide an insight into the postmortem findings.

MAF is a fentanyl analog, with replacement of the propionamide group by a 2-methoxyacetamide group. MAF is also closely structurally related to fentanyl (having an additional fluorine in the 2-position on the aniline ring structure). In Europe, MAF became available in late 2016. The first death in Sweden occurred on December 3, 2016. MAF was scheduled as a hazardous product prohibited for sale in Sweden on January 25, 2017, and later that year, on October 19, scheduled as a narcotic drug. This study reports the circumstances, postmortem findings, and toxicological findings in 11 deaths related to the intake of MAF.

Quantification of MAF in femoral blood was performed on an LC-30AD liquid chromatography system equipped with a Triple Quad 4500 mass spectrometer. Mobile phases A (0.05% formic acid in 10mM ammonium formate) and B (0.05% formic acid in methanol) were used at a flow rate of 0.8mL/min; the linear gradient was from 2% B to 100% B in 3.0min. An Acquity® BEH Phenyl column (60°C) was used. Electrospray in positive mode was used for ionization. Data acquisition with two transitions for MAF with  $m/z$  353.20/188.10 as quantifier and  $m/z$  353.20/105.10 as qualifier was used with D5-fentanyl as the internal standard ( $m/z$  342.0/188.0). A 0.5g aliquot of blood was fortified with internal standard and precipitated with 0.75mL of acetonitrile:ethanol (90:10) with the addition of 0.075% formic acid.

The 11 decedents were aged 27 to 41 years (mean 32.5), with one female and ten males. The manner of death was accidental in seven cases, natural disease in one case, and undetermined in three cases. The cause of death was intoxication by MAF alone ( $N=7$ ) or in combination with other drugs in all but one case, where death was attributed to acute complications of an underlying heart disease but with possible contribution from MAF. Significant postmortem findings that pointed toward opiate toxicity were lung congestion and lung and brain edema with a mean combined lung weight of 1,469 grams. Three subjects also presented with froth in the airways. There were few other significant findings, but pneumonia, hepatitis, and atherosclerosis of varying degrees were pathologies found. At least eight of the decedents had a history of drug abuse, and all but two were found dead indoors. One subject was found alive but in respiratory and cardiac arrest. At the hospital, the patient presented with RLS8 and an MAF concentration of 41ng/g. During hospitalization, the patient never regained consciousness and also developed acute kidney injury. The MAF concentrations in femoral blood ranged between 18–140ng/g with a mean of 47ng/g and a median of 34ng/g. In the literature, there are ten cases reported from the United States and three cases from Denmark where MAF was quantified in femoral blood. Concentrations were 22, 23, and 56 (ng/g) in the Danish cases and ranged between 0.21–39.9 (ng/mL) in those reported from the United States (mean 17.7ng/mL). Polydrug use was confirmed from toxicological analyses in all cases, but no other non-prescription opioids were present. Benzodiazepines (alprazolam, clonazepam, and diazepam) as well as the non-prescription norfludiazepam and etizolam were present, together with numerous other prescription drugs and the scheduled drugs amphetamine and tetrahydrocannabinol. At least three different routes of administration were suggested from the death scene findings: injection, oral administration of tablets, and snorting. The deaths occurred between December 2016 and May 2018, both prior to and after scheduling of MAF in Sweden.

The concentrations of MAF were in the upper concentration range compared to those previously reported in femoral blood and contribute to previous knowledge of fatal concentrations. Polydrug use was common but not necessarily an acute contributing factor. Since most deaths were accidental and unwitnessed, a possible risk factor is the use of MAF alone. The scheduling of MAF did not preclude its use.

### Methoxyacetylfentanyl (MAF), New Psychoactive Drugs, Fatal Intoxication



## K68 Homicidal Paraquat-Induced Respiratory Failure: A Case Report and Overview of Paraquat Testing in the Forensic Setting

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**Learning Overview:** After attending this presentation, attendees will recognize clinical findings, classic autopsy gross and histologic abnormalities, and the potential need for expanded toxicology testing in deaths due to paraquat toxicity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the importance of performing a complete autopsy and obtaining additional, non-routine specimens for toxicology testing during medicolegal autopsy examinations when the circumstances of death include suspected poisoning.

Paraquat is a water-soluble, highly toxic agent used in pesticides worldwide. Due to easy accessibility, it is commonly seen within the context of suicide in developing nations.<sup>1</sup> Complications of paraquat toxicity can mimic many respiratory diseases and be difficult to diagnose and treat, and subsequent mortality often ensues. Postmortem, pulmonary congestion, fibrosis, and glomerulosclerosis may be observed.<sup>2</sup> A combination of clinical history and findings at autopsy may suggest paraquat poisoning. However, both antemortem and postmortem testing of blood and urine can be negative. Preferential accumulation of paraquat in the lungs should be kept in mind, as paraquat testing of lung tissue can reveal the cause of death. Presented here is a medicolegal autopsy case of respiratory failure by homicidal paraquat poisoning after additional toxicology testing was performed on lung tissue.

A 69-year-old female who lived in Jamaica reported increased asthma-like attacks and generalized pain soon after she informed her husband that she wanted a divorce. Despite medical treatment, her symptoms persisted. One week later, she presented to the emergency room for worsening dyspnea and evidence of kidney and liver malfunction. Despite treatment for possible viral pneumonia with a superimposed bacterial infection, she ultimately died in the hospital due to respiratory failure, approximately one month after her initial symptoms. After death, the decedent's family was concerned that her husband might have poisoned her with paraquat (sold as Gramoxone® in Jamaica), given his increasingly abusive behavior. The decedent also messaged friends that her husband had made her food at the time she became sick, something that he had not done in years. Due to the allegations and clinical circumstances of respiratory failure over a relatively short period, a medicolegal autopsy was performed.

The autopsy was most significant for diffuse pulmonary fibrosis, a finding consistent with generalized lung injury that can be due to multiple etiologies. Review of the medical records included negative H1N1, influenza A, and influenza B testing. Paraquat testing on both antemortem blood and postmortem urine were negative. However, paraquat testing performed on lung tissue was confirmed at a concentration of 44ng/g by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Given these findings and the circumstances provided, the cause of death was attributed to complications of paraquat toxicity, and the manner of death was homicide.

Paraquat toxicity can manifest in a number of ways according to paraquat's accumulation within tissues. Depending on the dose, initial symptoms may be vague and easily confused with infectious diagnoses. Due to the small amounts ingested over time, this case displayed progressive dyspnea and persistent non-specific symptoms. Both negative findings and the lack of improvement with antibiotics and antivirals may guide the clinician toward other diagnoses. Furthermore, contextual information is essential. In this case, the decedent lived in Jamaica, where paraquat is more widely available than in the United States.

Knowledge of factors that can influence the pharmacokinetics of paraquat and other potential toxins such as solubility are critical in suspected poison-related deaths. Toxicology testing for paraquat performed on blood and urine may come back negative due to rapid uptake into highly perfused tissues, such as the lungs and kidneys.<sup>3</sup> Therefore, a negative result does not rule out paraquat toxicity. In this case, tissue samples were necessary to confirm paraquat as the causal agent. Furthermore, the use of a highly selective and sensitive analytical technique such as LC/MS/MS is required for a definitive determination of paraquat. LC/MS/MS is a preferred technique routinely used in the quantitative assessment of the severity of suspected poison-related intoxication. Though not routine, LC/MS/MS testing of lung tissue for paraquat is recommended if there is a strong suspicion of paraquat poisoning and diffuse lung injury is documented at autopsy.

### Reference(s):

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2. Sun B., Chen Y.G. Advances in the mechanism of paraquat-induced pulmonary injury. *Eur Rev Med Pharmacol Sci*. 2016;20(8):1597-1602.
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### Paraquat, Autopsy, Toxicology



### K69 Caught Looking: A High-Profile Vessel-Related Fatality in Miami, Florida

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**Learning Overview:** After attending this presentation, attendees will understand the role and importance of toxicology testing in the investigation of vessel-related fatalities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the importance of the toxicological investigation and the role of drug impairment in vessel-related fatalities.

According to the Boating Accidents Statistical Report issued by the Florida Fish and Wildlife Conservation and Commission, the State of Florida has averaged 56 vessel-related fatalities per year since 2009. Miami-Dade County, located in the southeastern part of the state, ranks high among the top ten Florida counties in which boating incidences occur. Miami is a city defined by water with boating and salt life very much a part of its culture. Whether fishing, snorkeling, scuba diving, or sunbathing, enjoying time on a boat is an everyday, year-round pastime that requires very little experience but offers an atmosphere conducive for “partying” and drinking alcoholic beverages. Unfortunately, this combination resulted in a high-profile fatality in Miami involving a Major League baseball player and two additional passengers.

The three men were known to be socializing and having drinks at a popular bar on South Beach. They left the establishment and boarded a 32-foot SeaVee® center console. The vessel began traveling from the Atlantic Ocean when it collided into a jetty as it attempted to enter the channel at a very high rate of speed. Autopsies completed determined blunt force trauma and drowning as the cause of death for all three occupants. The captain of the vessel had an iliac blood ethanol concentration of 0.147% and a cocaine and benzoylecgonine concentration of 0.126mg/L and 0.282mg/L, respectively. The second passenger had an iliac blood ethanol concentration of 0.065% and cocaine and benzoylecgonine of 0.077mg/L and 0.482mg/L, respectively. Both decedents with cocaine identified also had cocaethylene present at a level below the limit of quantitation (0.050mg/L) for the method. The third passenger had an iliac blood ethanol of 0.044% with only citalopram detected. The toxicology findings determined that ethanol and drugs were a factor in this boating accident. Overall, it was concluded that the vessel captain operated with his normal faculties impaired, in a reckless manner, at an extremely high rate of speed. The combination of ethanol and cocaine, at night, in an area with known navigational hazards such as the rock jetties and channel markers contributed to the fatal accident.

Toxicology testing is important in vessel-related fatalities for determining the presence or absence of impairing substances and can help in understanding the circumstances surrounding boating accidents. In this particular case, multiple factors were at play, including speed, darkness, navigational hazards, and impairment due to alcohol and drugs.

#### Boating, Vessel Fatality, Toxicology

## K70 Urinary Metabolites in Fatal Intoxications With Methoxyacetylfentanyl Could Indicate Time Until Death

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**Learning Overview:** The goal of this presentation is to increase attendees' knowledge of methoxyacetylfentanyl toxicology by providing urinary data in postmortem cases and discussing the potential value of such data in case interpretation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing knowledge of methoxyacetylfentanyl toxicology and providing data to facilitate interpretation of urinary findings in fentanyl analog intoxications.

Methoxyacetylfentanyl is a fentanyl analog in which the propionamide group is replaced by a 2-methoxyacetamide group. It contributed to 11 intoxications in Sweden between 2016 and 2018. In this study, urinary metabolites in eight fatal cases involving methoxyacetylfentanyl (all male, age 27–41) were analyzed. Femoral blood concentrations ranged from 17–140ng/g and the Cause Of Death (COD) was intoxication, except for one case listing COD as acute complications of an underlying heart disease but with possible contribution from methoxyacetylfentanyl. The hypothesis was that urinary metabolite profiles might correlate to the time between intake and death, potentially indicating a short period of abstinence before the last dose.

Urinary metabolites were analyzed by Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry LC/qTOF/MS (Agilent® 1290/6550) with and without hydrolysis with  $\beta$ -glucuronidase/arylsulfatase. Samples were diluted 1:4 in 1M sodium acetate buffer pH 5 and a negative urine sample was included as a control. The metabolites were separated on an Acquity® HSS T3 column (150 × 2.1mm, 1.8 $\mu$ m, Waters®) using a 13min gradient from 1%–40% acetonitrile with 0.1% formic acid in both solvents (60°C, 0.5mL/min). The method was not quantitative; but for individual analytes, peak areas generally correlate well with concentration. For confirmation of identified metabolites, methoxyacetylfentanyl was incubated with hepatocytes (5 $\mu$ M, 1 million cells/mL). Duplicate incubations for zero, one, three, and five hours were analyzed with the same method as the urine samples.

In total, 25 urinary metabolites were found, including eight glucuronides. Major biotransformations were O-demethylation, dealkylation to form the nor-metabolite, mono- and dihydroxylations of the phenethyl moiety (the latter with subsequent methylation), as well as combinations thereof. Methoxyacetylfentanyl was detected as the parent compound in all cases, and the most abundant metabolites in hydrolyzed urine samples included O-desmethyl-, O-desmethyl, phenethyl-hydroxy-, phenethyl-hydroxymethoxy-, and normethoxyacetylfentanyl.

When looking at the abundances of the parent compound methoxyacetylfentanyl, M21 (O-desmethyl) and M18 (glucuronide of M21) in urine, three distinct groups were observed. In three cases, the abundances of methoxyacetylfentanyl, M18 and M21 were all low (<380k, <21k, and <270k counts, respectively), which in combination with a substantial concentration in femoral blood (21–140ng/g) may indicate an acute intake while abstinent and a “rapid” death. In two of those cases, a syringe was found at the scene, suggesting intravenous administration and a rapid onset.

In two other cases, the abundances of methoxyacetylfentanyl (>2,400k) and M21 (>1,200k) were high while the abundance of M18 was low (<280k). This could indicate an acute intake while abstinent but a more delayed death where phase I metabolite M21 was formed and at least some phase II metabolite M18 was produced. Femoral blood concentrations were 17 and 31ng/g. In hepatocytes, M21 reached a plateau after 1h while M18 was still increasing at 5h of incubation.

In the remaining three cases, the abundances of methoxyacetylfentanyl (>3,000k) as well as both M18 (>2,300k) and M21 (>9,300k) were high, which could be indicative of an acute intoxication in a chronic user. (This group includes the case in which methoxyacetylfentanyl was a possible contribution to complications of heart disease.) Femoral blood concentrations were 18–51ng/g.

Based on eight cases, the major urinary metabolites of methoxyacetylfentanyl were identified. Differences in the abundance of methoxyacetylfentanyl and its major metabolites could be interpreted to indicate fatal intoxications in abstinent or chronic users. It is postulated that urinary concentrations of methoxyacetylfentanyl and two metabolites, in combination with the methoxyacetylfentanyl concentration in femoral blood, might be good indicators of the time between administration and death as well as prior use. However, to verify this, further (preferably quantitative) measurements of urinary methoxyacetylfentanyl, M18, and M21 in cases with well-established case histories are needed.

### Methoxyacetylfentanyl, Fatal Intoxication, Metabolites





## K71 Identification and Quantification of Classic, Prescription, and Synthetic Opioids in Hair by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Overview:** After attending this presentation, attendees will better understand how hair can be used as a matrix to identify and quantify different types of opioids, including fentanyl and derivatives, using Solid Phase Extraction (SPE) and LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a validated method for the confirmation of a comprehensive panel of opioids, including classic, prescription, and synthetic opioids, in hair. The current widespread misuse of both prescription and non-prescription opioids in the United States has reached an epidemic status. According to the United States Department of Health and Human Services, every day more than 130 people in the United States die after overdosing on opioids, and 2.1 million had an opioid use disorder in 2016. Drug testing tools to monitor opioid use and abuse habits in both the living and postmortem are necessary to understand the opioid use pattern, identify risk groups, and cause of death. Hair is becoming an alternative matrix of increasing interest in forensic toxicology to investigate drug use and abuse patterns due to its long window of detection. Hair growth rate is about 1cm/month; therefore, segmental hair analysis provides information of drug exposure from at least one to several months before samples collection.

**Hypothesis:** Highly sensitive and specific identification and quantification of a comprehensive panel of opioids in hair samples can be achieved utilizing SPE as a sample preparation and LC/MS/MS as an instrumental technique.

**Method:** This procedure allows for the confirmation of 27 opioids (8 classic and prescription opioids and 19 synthetic opioids) in 25mg of hair. After hair decontamination, the sample was pulverized using a beadmill. To 25mg of this powder, 15 internal standards (morphine-d3, codeine-d3, 6-acetylmorphine-d3, hydrocodone-d6, hydromorphone-d3, oxycodone-d3, fentanyl-d5, norfentanyl-d5, EDDP-d3, methadone-d3, oxymorphone-d3, tramadol-13C-d3, sufentanyl-d5, isobutyryl fentanyl-d5, and valeryl fentanyl-d5) and 2mL of digestion solution (0.01% formic acid + 2.5mM ammonium formate in water and methanol, 95:5, v/v) was added. The hair sample was digested overnight at 37°C in a heating block. The digestion was cleaned of interferences using a Phenomenex® Strata™-XC Strong Cation SPE cartridge. The elutants were dried under nitrogen and reconstituted using the initial mobile phase concentration (A:B, 95:5, v/v) containing 0.01% formic acid + 2.5mM ammonium formate (A) and acetonitrile (B) prior to LC/MS/MS analysis.

The chromatographic separation was performed in gradient mode employing a 100 x 2.1mm Kintex® C-18 analytical column with a 1.7µm particle size and mobile phases A and B. The gradient was programmed to run from 5% to 20% acetonitrile for 3min, 20% to 28% for 11min, 28% to 95% for 10min, then back to 5% for 1min, along with a 5min equilibration for a total run time of 30min. These chromatographic conditions were capable of resolving closely eluting isomers, such as 3-methylfentanyl, butyryl fentanyl, and isobutyryl fentanyl. The Shimadzu® mass spectrometer triple quadrupole LC/MS-8050 was employed in electron spray ionization positive mode, and two multiple reaction monitoring transitions were acquired per analyte.

The Limit Of Quantification (LOQ) was established to be 1pg/mg (3-methyl-fentanyl, 4-anpp, 4-methoxy-butyryl-fentanyl, acetyl fentanyl, acryl-fentanyl, alfentanil, butyryl fentanyl, carfentanil, EDDP, fentanyl, furanyl-fentanyl, isobutyryl fentanyl, MT-45, para-fluorobutyryl fentanyl, sufentanil, U-47700, and valeryl fentanyl), 10pg/mg (6-acetylmorphine, hydrocodone, methadone, morphine, norfentanyl, oxycodone, oxymorphone, and tramadol), or 100pg/mg (codeine and hydromorphone). The method was fully validated following the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. The parameters evaluated were linearity, limit of detection and LOQ, imprecision, accuracy, carryover, matrix effects, extraction efficiency, process efficiency, interferences, and autosampler stability.

**Conclusion:** A sensitive and specific method was developed for the identification and quantification of 27 classic, prescription, and synthetic opioids in hair by LC/MS/MS.

### Opioids, Hair, LC/MS/MS



## K72 Assessment of Postmortem Liver Samples Using a Validated Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Extraction for Fentanyl and Metabolites With Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Analysis

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**Learning Overview:** After attending this presentation, attendees will better understand a modified QuEChERS extraction combined with a sensitive method for determining concentrations for fentanyl and two known metabolites, norfentanyl and despropionyl fentanyl (4-ANPP), in postmortem liver specimens using LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by relaying a procedure that utilizes the unique properties of a QuEChERS extraction with modifications to allow for the extraction of fentanyl and metabolites from challenging postmortem matrices.

The opioid crisis in the United States has a serious impact on society. According to the National Institute on Drug Abuse (NIDA), more than 100 people die every day of opioid overdose. Even more concerning is the increase of overdose deaths involving synthetic opioids (e.g., fentanyl and fentanyl analogs), with increases from 3,105 deaths in 2013 to 28,466 deaths in 2017 (a 915% increase). In a forensic postmortem toxicological workflow, various specimens are often submitted for testing, including urine, blood, liver, kidney, and other tissues. For medicolegal death investigations, the interpretation of toxicological findings is a key component to establish the cause of death of an individual.

Common practices for tissue extractions involve Solid Phase Extraction (SPE) or Liquid-Liquid Extraction (LLE), but the process for these techniques can be time consuming and often involve tissue homogenization using blenders that could allow cross-contamination. In recent years, a QuEChERS extraction protocol was introduced in the market to deal with samples with a high content of fatty materials. By its nature, the liver contains many lipids that can contribute to matrix effects and be problematic for instrumentation. QuEChERS is ideal for tissue extractions due to its two-part process that allows for a direct extraction with organic solvent in the first stage, followed by a dispersive-solid phase extraction step. QuEChERS embraces the advantages of LLE, direct extraction from tissue with an organic solvent and cost effectiveness, combined with the advantages of SPE, reduction of matrix effects and impurities, without sacrificing selectivity or robustness.

In this study, the use of a modified QuEChERS extraction was demonstrated for testing a 0.2g liver sample. Homogenization was achieved using a Retsch® MM 200 high-speed mixer mill with homemade attachments for 1.7mL centrifuge tubes and 4.5mm stainless steel balls for pulverization, reducing the risk of cross-contamination by producing a homogenized sample in a single disposable tube. The quantification method was performed on an Agilent® 6470 Triple Quad Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) system. Chromatographic separation was achieved on a Zorbax® Eclipse Plus™ C18 RRHD 3.0x100mm, 1.8µm column with 0.1% formic acid and 5mM ammonium formate in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B).

The validation of a QuEChERS extraction protocol is presented in this study as an alternative analytical method for efficient extraction and detection of fentanyl and its primary metabolites, norfentanyl and 4-ANPP. The LC/MS/MS analysis was validated following the American Academy of Forensic Sciences Standards Board (ASB) standard 036 guidelines. The evaluated parameters include selectivity, matrix effects, linearity, bias, precision, processed sample stability, and proof of applicability using authentic fentanyl case samples.

The validation of the quantitative method for fentanyl, norfentanyl, and 4-ANPP was performed with a Limit Of Detection (LOD) of 0.1µg/kg and an Analytical Measurement Range (AMR) of 0.5 to 100µg/kg. Overall, the QuEChERS technique met all the acceptable criteria set by Matuszewski et al. with recoveries higher than 90.2%, matrix effects greater than 80.4%, and process efficiencies greater than 76.7% with %CV values below 12.8% for all target analytes.<sup>1</sup> The authentic case samples ranged in fentanyl concentrations from 56.6 to 462.3µg/kg with a mean of 149.2µg/kg ( $n=10$ ). The range of norfentanyl concentrations was 1.9 to 50.0µg/kg with a mean of 14.1µg/kg ( $n=10$ ). The range of 4-ANPP concentrations was 3.2 to 23.7µg/kg with a mean of 7.5µg/kg ( $n=7$ ).

The method was validated and the calibration curves reconcile well with forensic toxicology criteria. The extraction and LC/MS/MS method developed for the analysis of liver tissue for fentanyl, norfentanyl, and 4-ANPP is precise, sensitive, and reproducible at forensically relevant concentrations.

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### QuEChERS, Fentanyl, Liver



### **K73 A Food and Drug Administration (FDA) -Cleared Immunoassay Screen and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Confirmation for Benzodiazepines in Hair**

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**Learning Overview:** After attending this presentation, attendees will have learned the performance characteristics of an FDA-cleared immunoassay for benzodiazepines in hair, and the concentrations of selected benzodiazepines in hair samples after an extended aqueous wash. The concentrations are determined via LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing knowledge regarding methods useful for determining the concentrations of selected benzodiazepines in hair samples.

**Hypothesis:** Benzodiazepines could be detected in hair samples using a patented digestion method to remove the drug from hair, followed by a qualitative enzyme immunoassay screen. The benzodiazepine concentrations could then be determined quantitatively in hair using LC/MS/MS.

Hair samples were screened by: (1) drug extraction from hair using a patented method (United States Patent 8,084,215), and (2) performing an in-house developed microplate immunoassay using a selected monoclonal antibody with an oxazepam cutoff at 1ng/10mg hair and controls at -50/+100% of the cutoff. The immunoassay is designed for qualitative use and has been cleared by the FDA (k163590). Cross-reactivity of the immunoassay with selected benzodiazepines using oxazepam calibrator was alprazolam (277%), lorazepam (13%), diazepam (550%), nordiazepam (220%), oxazepam (100%), and temazepam (312%). The effects of cosmetic treatments (permanent wave, dye and bleach, relaxer, and shampoo) using benzodiazepine negative and positive samples in the immunoassay were determined. In this study, all negative hair samples remained negative after treatment, with all positive samples remaining positive after treatment. A comparison study ( $n=392$ ) of the immunoassay with LC/MS/MS results after an extended aqueous wash was conducted with stored samples previously received for workplace drug testing by the Psychomedics laboratory. All samples identified as negative by the immunoassay were confirmed as negative by LC/MS/MS ( $n=234$ ). One hundred thirty-four samples identified as positive by the immunoassay were confirmed positive by LC/MS/MS. Twenty-four samples identified as positive by the immunoassay were confirmed below the cutoff but containing drug above Lower Limit of Quantification (LLOQ) by LC/MS/MS after an extended aqueous wash.

The confirmation process consisted of a new hair aliquot that was first washed using an extended aqueous method followed by solid phase extraction and quantitation using an AB SCIEX™ API 3200 for LC/MS/MS confirmation using primary and secondary ions for each analyte in positive multiple reaction mode. The benzodiazepines confirmed were alprazolam, lorazepam, diazepam, nordiazepam, oxazepam, and temazepam. The cutoff for the confirmation was set at 0.2ng benzodiazepine/10mg hair. The LC/MS/MS method was linear from 0.05 to 20ng/10 mg with LLOQ of 0.05ng/10mg hair for all analytes. The LC/MS/MS method was reviewed and cleared by the FDA as a part of the benzodiazepine 510k process (k163590).

**Conclusion:** The first-of-its-kind, FDA-cleared, immunoassay screen and LC/MS/MS confirmation for benzodiazepines in hair has been presented. This study has provided the hair concentrations of six benzodiazepines analyzed by the assay.

#### **Benzodiazepines, Immunoassay, Hair**



## K74 Developing a Raman Microspectrophotometric Method to Quantitate Carboxyhemoglobin (COHb) in CO-Exposed Blood Samples

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**Learning Overview:** After attending this presentation, attendees will understand how Raman microspectrophotometry may be used to quantitate the COHb concentration of blood samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a novel method for COHb quantitation using Raman microspectrophotometry that is comparable with established spectrophotometric methods while eliminating the need for time-consuming sample preparation and limiting the use of consumables and the generation of waste.

COHb quantitation is a routine toxicological analysis performed in cases of acute carbon monoxide poisoning. Quick and easy-to-use methods of COHb quantitation include spectrophotometry and CO-oximetry, although these methods can be inaccurate and imprecise both at low concentrations and in putrefied postmortem specimens. More reliable gas chromatographic quantitation methods are available as well, but these require extensive sample preparation and, therefore, require elevated use of consumables and increased analysis time. The goal of this study was to develop a Raman microspectrophotometric COHb quantitation method and assess its precision and accuracy in estimating the COHb concentration of ten experimental unknown samples that were subsequently analyzed using an Ultraviolet (UV)-visible spectrophotometric method.

A Thermo Scientific™ DXR2 Raman microscope equipped with a 785nm laser was used to determine the COHb concentration of the samples analyzed for this study. Stock solutions of 0% and 100% COHb were prepared by bubbling ultra-zero air and carbon monoxide, respectively, through whole human blood (healthy living donor, gender not specified) containing K<sub>2</sub>EDTA anticoagulant for 30 minutes. A total of eight calibrator sets ranging from 0%–100% COHb at 10% increments were prepared via appropriate dilution of the stock solutions. These calibrators were deposited in 20- $\mu$ L aliquots onto aluminum foil-covered microscope slides, which were then subsequently dried in a fume hood for at least one hour prior to analysis. Spectra were acquired at ten separate locations on each sample, and these spectra were then averaged to provide a representative Raman spectrum of each sample. All spectra acquired were preprocessed in the OMNIC™ for Dispersive Raman software (version 9.8.372) using a sixth-order polynomial fluorescence correction prior to data analysis.

A strong linear correlation (*Average*  $R^2=0.98$ ) was found to exist between COHb concentration and the ratio of the peak intensities at approximately 1,552 $\text{cm}^{-1}$  and 1,580 $\text{cm}^{-1}$ . From the eight calibration curves analyzed, the limits of detection and quantitation were found to be 4.4% and 13.2% COHb, respectively. Intra- and inter-day studies of three calibrators (10%, 30%, and 50% COHb) indicated high precision, with the coefficient of variation ranging from 0.85%–4.26%. The accuracy of this method was evaluated through the quantitation of ten experimental unknowns, each analyzed in triplicate. Using the proposed Raman microspectrophotometric method, the true concentrations of seven of the unknowns were within the 95% confidence limits of the predicted COHb concentrations, with the percent error ranging from 2.00%–54.00%. The highest error was associated with unknowns having a COHb concentration at or below 10% COHb, below the limit of quantitation. The unknowns were subsequently analyzed using a Cary 3500 UV-Visible spectrophotometer, resulting in only six of the unknowns' true concentrations being within the 95% confidence limits of the predicted COHb concentrations, with the percent error ranging from 6.86%–77.20%. In this case, the highest error was associated with an unknown that had been correctly quantitated via the Raman microspectrophotometric method with a percent error of 3.60%.

This study demonstrates that Raman microspectrophotometry may be used to quantitate the COHb concentration of blood samples. Furthermore, this method is comparable with established spectrophotometric methods while eliminating the need for time-consuming sample preparation and limiting the use of consumables and the generation of waste.

### Carbon Monoxide, Carboxyhemoglobin (COHb), Raman Microspectrophotometry



## K75 EtG/EtS in Ethanol Negative Urine Specimens From Sexual Assault Victims

*Kristin W. Kahl, MS\*, University of Miami Toxicology Laboratory, Miami, FL 33136; Lisa J. Reidy, PhD, University of Miami Forensic Toxicology Laboratory, Miami, FL 33136*

**Learning Overview:** After attending this presentation, attendees will understand the significance of urine Ethyl Glucuronide/Ethyl Sulfate (EtG/EtS) testing in sexual assault cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing data demonstrating the usefulness of EtG/EtS urine quantitation from victims of sexual assault where alcohol is indicated but ethanol is not detected.

EtG and EtS are minor metabolites of ethanol that have traditionally been used as biomarkers of ethanol use in abstinence programs due to their longer half-lives relative to ethanol. The longer half-lives of these metabolites may also be useful for crimes where reporting and sample collection times may vary, such as Drug-Facilitated Crimes (DFC). The classification of a DFC includes Drug-Facilitated Sexual Assaults (DFSA). According to literature, ethanol is the most frequent drug detected in DFSA victims; however, due to the variation in reporting and sample collection times, some victims may not have blood or urine collected until hours or days after the alleged incident. Delays in sample collection can result in specimens being negative for ethanol, even if the victim was intoxicated at the time of the incident. This study investigates whether EtG and EtS are useful markers of ethanol use in DFSA cases where urine specimens are negative for ethanol.

Urine specimens utilized in this study were from suspected DFSA victims submitted to the University of Miami Toxicology Laboratory (UMTL) from June 2018 to July 2019. Specimens were first analyzed for ethanol using Headspace/Gas Chromatography/Flame Ionization Detector/Mass Spectrometry (HS/GC/FID-HS/GC/MS/FID) with a Limit Of Detection (LOD) of 0.01g/100mL. If ethanol was not detected in a urine specimen and alcohol was indicated from case histories, the specimens were analyzed for EtG and EtS by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). The quantitative EtG/EtS method was validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) validation guidelines.

The quantitation range for EtG was 200–10,000ng/mL (LOD 100ng/mL) and 50–2,500ng/mL for EtS (LOD of 25ng/mL) with a 1/x linear model. No significant matrix effects or carryover were observed. A dilution study was also performed during validation and case specimens were diluted, when necessary. Due to EtG being produced without the ingestion of ethanol, EtG is only reported if EtS is also present in the urine specimen. Case information and history were taken from sexual assault cases forms. The quantitative results, ratio of EtG:EtS, and case information were reviewed for each case.

Between June 2018 and July 2019, 167 urine DFC cases were submitted to the UMTL, of which 70 specimens were tested for EtG/EtS. Neither EtG nor EtS was detected in 12 of the specimens. Sixty-four cases directly stated, or indicated, that alcohol may have been involved in the incident, but no ethanol was detected. Of the cases in which alcohol was indicated, EtG and/or EtS was reported in 57 (89%) of the cases. The concentration of EtG ranged from 212ng/mL–467,650ng/mL and 60–117,650ng/mL for EtS. In seven cases, both EtG and EtS or only EtS were detected but were less than the lower limit of quantitation. The ratio of EtG:EtS ranged from 0.84–12.96 (mean: 3.85, median: 2.67). The range of time elapsed from the time of the incident to sample collection was 7–137h. Seven specimens were collected within 0–12h, 24 specimens were collected within 13–24 h, 16 specimens were collected within 24–48 h, and 23 specimens had unknown collection times or were collected more than 48 hours after the alleged incident.

Although testing for EtG/EtS in DFSA cases is not considered routine, the results presented show a high level of consistency between self-reported alcohol use and the detection of EtG/EtS in urine specimens when ethanol is not detected. The results also show that although sample collection times after the alleged incident can vary, EtG/EtS are still able to be detected for multiple days after ethanol ingestion, thereby corroborating the victim's statement of alcohol use.

### Sexual Assault, Ethyl Glucuronide, Ethyl Sulfate



## K76 Postmortem Pediatric Forensic Toxicology

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**Learning Overview:** After attending this presentation, attendees will have gained an appreciation for the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both a general and case-specific sense.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 20<sup>th</sup> Annual Special Session within the Toxicology section, pediatric cases involving toxicological findings are discussed. As a relative dearth of interpretive information involving toxicological findings in the pediatric population exists, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation or issue-specific concern, including pharmaco- toxicokinetic data and other relevant ancillary information, followed by audience participation to provide interpretive clarity around case-specific impacts of the toxicological findings. This session, attended by various sections of the Academy, allows for various perspectives of case issues that lead to integrative consensus, or differing opinions, as to cause of death in children.

Four cases will be presented that highlight the difficulty in assessing the role of toxicants in each case or the lengths one must go to in some cases to reach some interpretive value. Chris Milroy, MD, LLB, Andrew Baker, MD, Loralie Langman, PhD, and Robert A. Middleberg, PhD will be reviewing cases from their experience as forensic pathologists and toxicologists that highlight the issues and confounders in the pediatric population.

Dr. Milroy will be discussing a case involving diphenhydramine with an interesting twist. While cases involving diphenhydramine in young children have been reported and summarized in postmortem literature, each case presents challenges due to the pharmacological nature of the substance and other potential co-morbidities.

Dr. Baker will focus on an unresolved case involving an elevated sodium finding in vitreous humor in a 6-year-old. He will explore possible explanations given the case history and seek audience input to further case closure.

Dr. Langman will introduce a case of *in utero* death. As these cases are particularly challenging in respect to toxicological findings, continued discussions on this subject matter will hopefully bring some clarity to case findings in these deaths.

Dr. Middleberg will present a case of a 14-year-old female who was tortured and killed by her mother and mother's boyfriend. The dismembered body was found to contain what appeared to be high concentrations of diphenhydramine. Questions surrounding the findings were significant in respect to manner and cause of death and the ultimate outcome of the case.

### Pediatric, Toxicology, Postmortem



### LW1 The Murder of Matt Warren: Father of Former Alameda County Prosecutor, Three-Time California Governor, and Supreme Court of the United States (SCOTUS) Chief Justice Earl Warren

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**Learning Overview:** After attending this presentation, attendees will learn about the investigation of an unsolved murder from 1938. Attendees will learn about the investigative techniques of a high-profile murder case of the time and will also learn how modern investigative methods can be applied to a crime scene from more than 80 years ago.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing knowledge when reviewing historical evidence in the form of documents and crime scene photographs. Attendees may catch a glimpse into the thinking of a famous political figure and how the death of his father may have influenced his decisions as Chief Justice of the Supreme Court.

On May 14, 1938, Methias Warren, father of former United States Supreme Court Justice Earl Warren (*Brown v. Board of Education*), presidential candidate, three-time Governor of California, and chair of the infamous commission investigating the assassination of John F. Kennedy (JFK) that bears his name, was found bludgeoned to death in his home at 707 Niles Street in Bakersfield, CA. Many theories regarding the identity of the killer and the motive were asserted. Many argued that the assailant was most likely a transient. However, the evidence points to other plausible motives. There were many other “whodunit” theories, and, to this day, the case remains an unsolved mystery.

Eighty years later, the Historical Research Center on the campus of California State University, Bakersfield accessioned the records of the Kern County Superior Court. Buried within this body of records is information that sheds light on this cold case. Records include police reports, crime scene photographs, interviews, autopsy photographs, coroner’s inquest, and newspaper articles of the day. A “dream team” of investigators was assembled representing multiple law enforcement agencies across the state of California. These were the “crack” investigators of the day. Newspapers of the day published detailed descriptions of the crime scene. Readers even had glimpses into the published photographs of the crime scene. Yet, the case remains unsolved, even when the “third degree” was employed while questioning suspects. Tensions developed between the local law enforcement officials charged with investigating the crime and the handpicked investigators brought in by Warren.

While limited forensic science applications were applied in this case, the evidence recorded at the time was such that those techniques could have been employed. Techniques such as latent fingerprint development, footwear examination and comparison, and bloodstain pattern analysis are but a few that might have been employed at that time. This begs the question: What effect did not knowing who committed the murder of his father have on the chief justice? Could a case decision that resulted in the issuance of the Miranda warning or his desire to determine what happened in the assassination of JFK been a result of his anxiety? What could have turned a staunch Republican into the overseer of what was considered a liberal Supreme Court? This presentation will discuss this evidence, reflecting upon the investigative techniques of the time, the political implications, and brief bloodstain pattern analysis of the crime scene based upon evidence photographs.

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#### Warren, SCOTUS, Murder



### LW2 Old Shaky: The C-124 Globemaster

*James McGivney, DMD\*, Saint Louis, MO 63119*

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**Learning Overview:** After attending this presentation, attendees will understand the role of the C-124 Globemaster in the aviation history of the United States military and its involvement in a number of mass disasters.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by detailing the history of the C-124 Globemaster.

Back in the day, the C-124 Globemaster was the largest military transport ever manufactured. The plane had two decks and could carry 200 fully equipped soldiers or serve as a hospital transport for 124 patients on litters along with their attending staff. As a cargo hauler, it could carry tanks, guns, trucks, and other heavy equipment. It was the only aircraft of its time capable of transporting heavy equipment, such as tanks and bulldozers, without disassembling the equipment.

The plane did not see any service in World War II but was developed from a prototype of the World War II-era Douglas C-74 Globemaster and from lessons learned during the Berlin Airlift. The C-124's design featured two large clamshell doors and a hydraulically actuated ramp in the nose, as well as a cargo elevator under the aft fuselage.

The first deliveries of the Globemaster began in May 1950 and continued until 1955 with a total of 448 aircraft. The C-124 was operational during the Korean War and was also used to assist supply operations for Operation Deep Freeze in Antarctica. The aircraft performed heavy lift cargo operations for the United States military worldwide, including flights to Southeast Asia, Africa, and elsewhere. From 1959 to 1961, they transported Thor missiles across the Atlantic to England. The C-124 was also used extensively during the Vietnam War transporting materiel from the United States to Vietnam. Until the C-5A became operational, the C-124, and its sister, the turboprop C-133 Cargomaster, were the only aircraft available that could transport very large loads.

The plane was powered by four Pratt & Whitney piston engines-rated at 3,800hp that could push it through the air at a speed of 320mph, at a range of almost 2,200 miles. It could carry 216,000lbs., maximum.

“Old Shaky’s” name derived from the large Pratt & Whitney piston engines, which had cylinders the size of coffee cans. The noisy conglomeration of connecting rods, push rods, and pistons made for a deafening roar and a shaky plane ride.

The Pratt & Whitney R-4360 Wasp Major was the largest mass-produced aircraft piston engine ever made. It was a 28-cylinder, 71-liter, 56 sparkplug, air-cooled radial monster. It was initially supercharged. The final evolution of the Wasp Major incorporated a turbocharger as well.

The plane was involved in several noteworthy accidents. In 1951, a Globemaster ditched in the Atlantic Ocean off of Ireland due to a cargo fire. Initially, a search plane spotted men in life rafts, but when rescue vessels arrived on scene, no survivors or rafts were found.

In November 1952, a C-124A that left McChord Air Force Base in Washington state crashed into the Colony Glacier on Mount Garrett, some 40 miles east of Anchorage, AK. All 41 passengers and 11 crew members were lost. Sixty years later, debris from the plane and remains of some of the victims were found by the Alaska National Guard on June 10, 2012, having apparently been uncovered due to the receding of the glacier. By 2014, remains of 17 victims had been recovered and identified by the Defense POW/MIA Accounting Agency (DPAA).

On June 18, 1953, a Globemaster took off from Tachikawa Air Base in Japan. Shortly after takeoff, one of the engines failed, forcing the pilot to make an emergency landing. Due to a loss of airspeed, the pilot lost control and crashed into a melon patch, killing all 7 crew members and 122 passengers. At the time, these 129 fatalities constituted the worst accident in aviation history and would not be exceeded until a midair crash over New York City in 1960.

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### Aviation, Globemaster, Mass Disaster





### LW3 Murder of the Schoolmarm: The First School Shooting

*Alexander Jason, BA\*, ANITE Group, Pinole, CA 94564*

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**Learning Overview:** The goal of this presentation is for attendees to become familiar with techniques of shooting incident reconstruction and evidence collection used in a historic 1850 shooting incident.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how firearms evidence was utilized in an early (1850) shooting reconstruction and how the same techniques can be currently utilized to perform a shooting reconstruction.

This incident was an early documented shooting reconstruction and from historical research it appears to be the first school shooting in the United States.

In 1850, the local schoolmarm was fatally shot as she attempted to unlock the one-room schoolhouse early one morning. The weapon was a shotgun. The fatal wounds were in the left side of her head where pellets had penetrated. There were also pellet strikes on the schoolhouse door, which was behind her. There were no witnesses, but one key piece of evidence was a shotgun wadding found between a nearby tree and the schoolhouse. The wadding was a small section of paper that, after an investigation by the local sheriff, incriminated a young man who was charged with murder. A basic shooting reconstruction was performed by placing a broom straw into one of the perforating holes in the schoolhouse door. This straw pointed back toward a location near a tree where footprints were found in the dirt, which also implicated the suspect. Although the suspect denied involvement in the shooting, key evidence was recovered from his clothing that proved to be of high probative value. The suspect was charged with the murder and a trial was held.

The historical official records of the trial were obtained and the details of this incident made possible a review of the investigation and the trial testimony, which was hand-recorded in the courtroom. The trial resulted in the defendant being found guilty and sentenced to death by hanging.

A key element in the trial was examined in this study's experiment utilizing a similar black powder shotgun and newspaper. High-speed videography was used to show several aspects of the projectiles and wadding dynamics. The purpose of the experiment was to determine the possibility that paper used as wadding would not be burnt or destroyed during the black powder ignition. This presentation will show how the trajectory reconstruction was performed and how the paper wadding was used to convict the suspect. A video demonstration and re-enactment will be utilized to demonstrate the operation of the double-barrel black powder shotgun used in the incident and how the paper wadding was identified to the suspect.

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### Shooting Reconstruction, Shotgun, School Shooting



### LW4 To Infinity and Beyond: E.T. at the Crime Scene and the Forensic Challenges of the Current Era

Matteo Borrini, PhD\*, Liverpool John Moores University, Liverpool, AE L3 3AF, UNITED KINGDOM

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how modern multicultural society represents a challenge for forensic investigators.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing examples of the problems offered to the investigators by the current multicultural environment and movements of populations.

The history of the American Academy of Forensic Sciences (AAFS), established in 1948, and that of the so-called modern ufology that begins with the Kenneth Arnold “flying saucer” sighting on June 1947, are almost contemporary. However, they are two completely different worlds: the first based on the rigorous application of scientific principles for legal purposes, the other on the use of a (pseudo)scientific method to search evidence of contacts with intelligent entities from other planets.

But what would happen if we cross borders and combine these two worlds? What would happen, in a hypothetical scenario, if E.T. crossed the boundaries of the galaxy and interacted with the human world?

In previous AAFS scientific meetings, it has been demonstrated how topics apparently far from the forensic world may instead represent food for thought in the investigation of ancient beliefs, of historical murders, or ritual practices.<sup>1-4</sup> In addition, these unusual topics can provide a springboard for teaching and dissemination of forensic sciences.<sup>5</sup>

The approach to the ufological world is aligned with the intention of the previous presentations, but also offers a further opportunity of analysis on the theme of “other” populations. Currently, millions of people interact and cross borders, creating multiethnic nations. This scenario represents a new and greater challenge for forensic practitioners, especially for those (e.g., anthropologists) involved in the identifications of unknown individuals.

The examination of the supposed alien abduction cases and the related testimonies under regressive hypnosis is an excellent example of the dangerous use of some interview techniques. These reports are also a fertile ground for the evaluation of eyewitness statements, offering the possibility to study the mechanisms of (false) memories. It is known how testimonial evidence can create difficulties in the reconstruction and interpretation of criminal events if recorded without well-established protocols.

In addition, the critical analysis of the so-called “alien autopsy video” related to the supposed Roswell Unidentified Flying Object (UFO) crash is useful for both teaching and scientific purposes. The examination of the film allows not only the debunking of this video, but also the opportunity to analyze and discuss the evolution of autopsy techniques, particularly in contexts of Disaster Victim Identification (DVI) and mass disasters.

Finally, the cute alien E.T. and all the creatures from other planets depicted in science-fiction movies are metaphors of the problems related to foreign individuals that cross the borders without documents. If people travel without leaving a trace of their movements in the country of origin and arrival, they become vulnerable subjects who may be involved in crimes as victims or offenders. The lack of data makes their identification and protection difficult and sometimes impossible.

Although aliens have never crossed the galaxy’s borders to reach our planet, their fictional stories can inspire the forensic community to cross the boundaries of science to develop new strategies to guarantee protection and safety for everyone in new, multicultural societies.

The goal of this presentation is to provide an opportunity to discuss some of the forensic aspects related to a new reality, where individuals increasingly move between countries, and the forensic community is called upon to guarantee the safety and security of everyone.

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#### Multicultural Society, Forensic Sciences, Ufology



### LW5 Leonhard Euler's Mysterious Blindness and Fever

*John David Bullock, MD, MPH\*, Winchester, MA 01890; Harrison B. Hawley, MD, Miami Valley Hospital, Dayton, OH 45409-2902; Ronald E. Warwar, MD, Warwar Eye Group, Dayton, OH 45409*

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**Learning Overview:** The goals of this presentation are for the attendee to: (1) learn how to analyze historical data in order to make a retrospective diagnosis; and (2) learn about brucellosis

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining how to analyze historical/biographical data in order to diagnose distant events/conditions.

The renowned Swiss polymath Leonhard Euler (1707-1783; spent the vast majority of his adult life at the St. Petersburg Academy of Sciences in Russia) is often grouped along with Archimedes, Newton, Gauss, and Riemann as one of the five most famous mathematicians of all time and the most prolific. He produced 886 papers and books and is honored by 96 eponymous mathematical terms. He introduced the concept of a function,  $f(x)$ , the modern notations in trigonometry, and the mathematical symbols:  $e$ , for the base of the natural logarithm;  $\sigma$ , for summation; and  $i$ , the imaginary number representing the square root of minus 1. He popularized  $\pi$ . He derived "Euler's identity,"  $1 + e + i + e^i = 0$ , which has been called the "most remarkable formula in mathematics" by Nobel Laureate Richard Feynman, and he was the first to present Newtonian dynamics in mathematical form. He has been honored by stamps, currency, a Paris street, and a lunar crater.

In 1735, at age 28, he developed an inexplicable and near-fatal febrile disease that spontaneously remitted. A mezzotint copy by Vassili Sokolow of a (now lost) portrait of Euler in 1737 by Johann Georg Brucker shows a normal physiognomy and appearance of both eyes. The following year, Euler suffered a relapse of his febrile illness and began to lose the sight of his right eye associated with what was described as an "abscess" (most likely, septicemia causing a septic cavernous sinus thrombosis with ischemic optic neuropathy). His fevers continued. A 1753 portrait by the celebrated Swiss painter Jakob Emanuel Handmann shows a ptotic right upper eyelid with slight right miosis (consistent with a right Horner's syndrome), a right hypertropia (consistent with a right fourth cranial nerve palsy), and normally appearing anterior ocular segments. In 1766, Euler developed a cataract in the left eye, the unsuccessful 1771 extraction of which left him virtually completely blind, but still able to perform highly complex mathematical calculations in his head. He then continued his prodigious output of mathematical discoveries to an even greater extent. In 1783, Euler suddenly developed a fatal brain hemorrhage, most likely the result of a ruptured intracranial aneurysm.

Today, common causes of recurrent fever include immunizations, viral infections, heat exhaustion, chronic inflammation, malignant tumors, antibiotic, hypertensive or seizure medications, and certain bacterial infections, including, but not limited to, tuberculosis, typhoid fever, epidemic typhus, or tertiary syphilis, all of which are either incorrect or extremely unlikely.

For centuries, an essential part of the Russian diet has been raw milk, the consumption of which is a significant risk factor for brucellosis, the chronic debilitating febrile illness attributed to the pioneer nurse Florence Nightingale after her return to the United Kingdom from the Crimean War in 1856. Brucellosis, also known as undulant or Malta fever, is a highly contagious, and the most common, zoonosis, is caused by the ingestion of unpasteurized milk, cheese, and other dairy products or undercooked meat from animals (usually sheep or goats) infected with a small, gram-negative, non-motile, non-spore-forming, non-encapsulated, rod-shaped coccobacillary bacteria that functions as a facultative intracellular parasite, causing a chronic disease, usually persisting for life. This organism, eponymously designated *Brucella spp.*, was first noted in a splenic autopsy specimen by the Scottish pathologist and microbiologist Sir David Bruce. The Mediterranean Fever Commission of 1904–1906, chaired by Bruce, traced its transmission to the unpasteurized milk of Maltese goats.

An exhaustive literature review strongly suggests that, given the history of an acute, febrile illness that progresses to a chronic disease with ophthalmic and neurological complications (CN IV palsy, Horner's syndrome, and optic neuropathy), and having the possible terminal event being rupture of a mycotic intracranial aneurysm, the most likely diagnosis to explain this complex set of circumstances regarding the blindness and death of Leonhard Euler is undulant fever, progressing to neuro- and ocular-brucellosis, and death by rupture of a *Brucella*-infected cerebral aneurysm.

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### Euler, Brucellosis, Fever

### LW6 Forensic Photo Analysis: Who, What, When, Where, Why?

Colleen M. Fitzpatrick, PhD\*, Fountain Valley, CA 92708

**Learning Overview:** By attending this presentation, attendees will learn how multidisciplinary analysis can be applied to solve a mystery, based on the example of this picture of an old barroom, taken in the late 19<sup>th</sup> or early 20<sup>th</sup> century.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the value of a multidisciplinary approach for extracting an assortment of details that can nevertheless be used in a coherent manner to solve a mystery based on the questions who, what, when, and why.



There was no *a priori* information about the photo except for an address on the back—805 Baronne Street, apparently a location in the New Orleans, LA, business district. Yet through careful observation, it has been possible to determine the Global Positioning System (GPS) coordinates of the photographer, the date the picture was snapped to within a few weeks, and the likely occasion for which it was taken.

Historically, the date of the picture must be prior to October 1919 or after April 1933—either before or after Prohibition. There are many items in the photograph, both obvious and not so obvious, that could assist in narrowing the date further. The Falstaff® beer sign, the Coors® beer pennant, the cash register, even the pictures on the wall behind the bar (actually advertisements for alcoholic drinks) are useful in indicating a date of about 1910 when all of those products were simultaneously on the market.

However, the most valuable items for dating the picture are not in the bar, but are seen outside the bar, specifically the car that is visible through the open door, parked under a sign on a shop across the side street. The car can be compared to vintage catalogs to identify it as a 1911–1912 Ford® Roadster®, which provides an earliest date as October 1, 1910, the date the first Roadster® rolled off the production line.

The sign is more difficult to analyze, as only parts of the lettering are visible. However, using old city directories, the mystery of the sign and the identity of the bar's owner can be solved. Beginning in 1912, the bar belonged to Paul Dimaggio, the great-grandfather of the picture's owner. He is listed at that address until 1919, when he probably closed the bar because of the ban on alcohol sales. With the assistance of modern image processing, the writing on the sign can be deciphered to read "Goreau Plumbing."

The only problem with this is that, according to the city directories, the Goreau Plumbing Company moved from that location in 1909—at least that is the last year it is listed at that address. In 1910, the address is absent from the directory, and in the 1911 directory, the building across the street was occupied by the Southern Packing Manufacturing Co. So how is it possible that the Goreau sign appears in the background of a photograph of Paul Dimaggio, even though he does not appear in the directory on Baronne Street until 1912, long after the Goreau's were gone?

Using city directories and maps, it has been possible to determine eight events that had to happen in a specific order to create the scene in the picture with Paul DiMaggio standing in his bar with the sign for the Goreau Plumbing Company visible across the side street. There is only one place the photographer could have been standing, and there is likely only one occasion for the picture.

### Forensic, Photograph, Analysis



## Young Forensic Scientists Forum—2020

### Y1 Exploring Variation in the Human Nasal Bones Using Elliptical Fourier Analysis (EFA)

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**Learning Overview:** After attending this presentation, attendees will better understand the complex morphology of human nasal bone shape and the effects of ancestry, sex, and age at death on the observable variation in this skeletal region. The learning objectives include demonstrating the usefulness of EFA to describe complex skeletal shapes prior to constructing predictive models for use in a forensic setting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding to a growing body of knowledge regarding the range of normal variation present in the upper face.

Forensic anthropologists study skeletal variation in order to make predictions regarding the ancestry, sex, and age at death of unidentified remains for the medicolegal system. Ancestry determination is an important aspect of biological profile construction as this information is often included in missing persons reports. Ancestry is determined from the skeleton by utilizing both quantitative and qualitative trait assessments. While qualitative trait definitions allow for the inclusion of nuances in shape variation not easily captured through quantitative approaches, qualitative traits are inherently problematic because of their reliance on observer experience and extreme trait depictions. This pilot study uses EFA to more precisely capture the complex shape variation in the upper facial skeleton. EFA reduces the subjectivity in assessing qualitative traits, allows for the recognition of small nuances in shape, and allows for a precise determination of the influence of relevant independent variables, such as sex and ancestry, on that shape variation.

This study utilizes a sample of 97 Euro-American and 42 Afro-American individuals from the Bass Collection at the University of Tennessee, Knoxville. The nasal bones were photographed with a Nikon® D3200 DSLR camera and an 18–55mm lens following a strict alignment protocol. The digital images were then uploaded into Photoshop® v.17.1 and edited so that only the outlines of the isolated nasal bones remained. These nasal bone renderings were then uploaded into SHAPE™ v.1.3 and subjected to elliptical Fourier and Principal Component (PC) analysis. The following null hypotheses were tested with Analysis of Covariance (ANCOVA) using the principal component scores as dependent variables: (1) nasal bone shape is not influenced by ancestry, (2) nasal bone shape is not influenced by sex, (3) nasal bone shape is not influenced by age at death, and (4) nasal bone shape is not influenced by the interaction between sex and ancestry.

The first four effective PCs (PC1 to PC4) account for ~95% of the total variation in nasal bone shape and were subsequently examined in detail. These PCs describe, respectively: nasal bone height relative to breadth, frontonasal suture breadth relative to nasal tip breadth, nasal bone symmetry, and overall nasal bone breadth. The vast majority of the systematic variance in shape (~80%) is described by PC1, with only a small amount (~15%) being accounted for by the other three PCs combined. In the ANCOVAs, relative nasal bone height is significantly influenced by sex, ancestry, and the interaction between these two variables, with the interaction being the most important: on average, Afro-American females exhibit taller nasal bones relative to width than their male and Euro-American counterparts. Frontonasal suture breadth relative to tip breadth (PC2) is affected by sex and especially the interaction: Afro-American males exhibit relatively broader nasal tips than the other groups. Nasal asymmetry (PC3) seems to vary randomly, not being affected by any of the independent variables in the ANCOVAs. Finally, overall breadth (PC4) is affected by ancestry and age when controlling for nasal bone length: Afro-Americans and older individuals tend to display broader nasals than Euro-Americans and younger individuals.

This study illustrates the complex nature of nasal bone variation, as ancestry, sex, their interaction, and age all significantly influence nasal bone shape in specific circumstances. While nasal bone shape is often used in a forensic context to determine ancestry, this study demonstrates that the interaction between ancestry and sex contributes more to that variation than either ancestry or sex alone. Furthermore, the percentage of variation in shape explained by all PCs amounts to less than 2%, indicating that over 98% of the variation observed in nasal bone shape cannot be explained by sex, ancestry, age, and/or the interaction. Therefore, more sophisticated research is needed before accurate predictive models can be created utilizing this anatomical region.

#### Elliptical Fourier Analysis, Human Variation, Nasal Bones



## Young Forensic Scientists Forum—2020

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### **Y2 The Efficiency of a DNA Database for Sexual Crimes by Its Victim-Offender Relationship: A Portrayal From Central Brazil**

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**Learning Overview:** After attending this presentation, attendees will understand how DNA databases are extremely useful in investigating sexual crimes and will see their efficiency in the victim-offender relationship.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing, through the results obtained in this research, that the hypothesis of the “no need to use DNA databases to investigate sexual crimes” is totally refuted. In addition, the results show greater elucidation of sexual offenses of unknown perpetrators.

Despite the technological advances, there is a growing accumulation of sex crimes evidences stored without being tested for DNA for many reasons. One of these reasons is the idea that most of these crimes are committed by known offenders, thus the law enforcement agencies did not view DNA evidence as helpful or necessary to the task of investigating a reported crime; with the consequence being there was no indication of suspects for confrontation. However, with the advent of DNA databases, this evidence has been seen as valuable sources of information for investigations. International studies indicate that DNA typing can be useful for both known and unknown assault cases. In Brazil, there is no study with this approach, and its implantation of DNA databases is recent. Thus, the objective of this study was to evaluate the potential of the DNA Database of the Estate of Goiás (Brazil) in the elucidation of sex crimes by its victim-offender relationship.

The samples were submitted to DNA extraction by differential lysis with the PrepFiler® Express™ Forensic DNA Extraction kit and the AutoMate Express™ system, followed by amplification of Short Tandem Repeat (STR) autosomal genetic markers with GlobalFiler®, capillary electrophoresis in the ABI® 3500 genetic analyzer, and by the insertion in the DNA Database of State of Goiás using the Combined DNA Index System (CODIS) 7 software. The sample's rate was evaluated with profiles eligible for insertion and coincidence rates according to the victim-offender relationship and for the statistical approaches the program EpiInfo™ v. 7 was used.

A total of 2,165 cases were reviewed, of which 286 had information regarding victim-offender relationships. After DNA typing, 192 were inserted in the DNA Database of State of Goiás, of which 71 were related to known aggressors, 121 to stranger aggressors, and genetic coincidences were detected only with cases of unknown aggressors, involving 41 cases. Thus, the hypothesis of the “no need to use DNA databases to investigate sexual crimes” is totally refuted. In addition, the results indicated greater elucidation of sexual offenses of unknown perpetrators. Due to the limited body of studies with this approach in Brazil, the Brazilian DNA Databases have only been used for a few years and with its characteristics of legal basis, it can be stated that the greater elucidation of sexual offenses of unknown perpetrators is a current picture that can be modified according to the evolution of the Brazilian DNA database's growth. Thus, future research should be performed for a reliable view. Currently, it is understood that this tool cannot be ruled out for crimes of non-stranger aggressors.

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#### **DNA Database, Sexual Crimes, Victim-Offender Relationship**



## Young Forensic Scientists Forum—2020

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### Y3 The Effect of Human Decomposition on Fired Bullets and the Implications for Identification

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**Learning Overview:** After attending this presentation, attendees will understand the effects of human decomposition on individual characteristics in land impressions on bullets and how the deterioration over time affects an examiner's ability to analyze the bullets.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a novel analysis of an examiner's ability to identify the source weapon in cases involving bullets found in decomposed human remains.

For the purpose of this study, copper full metal jacketed bullets were placed in various regions of the body in two donors (Donor 1 and Donor 2) at the Northern Michigan University Forensic Research Outdoor Station (FROST) during the summer of 2019. Two bullets were placed in each of the selected regions so one could be left *in situ* during the entire data collection period and one could be repeatedly removed and replaced to assess the effects of decomposing soft tissue at shorter time intervals. All bullets were test fired from the same firearm into a standard tank at the Michigan State Police Detroit Laboratory and one control bullet was retained for comparison against the study bullets.

The condition of the donors and the other research in which they were participating affected the researchers' decisions regarding bullet placement. Prior to bullet placement, Donor 1 was in an advanced state of decomposition, meaning decomposition in some areas of the body was too far advanced for them to be viable placement locations. Donor 1 bullets were placed in the eye sockets, abdominal cavity, resting on the left femur, and in the soil near the body. Prior to bullet placement, Donor 2 was in an early stage of decomposition, but was part of an ongoing study focusing on gut bacteria, which precluded placement of bullets in the abdomen. Donor 2 bullets were placed in the right and left arms and resting on the left femur.

When the study bullets were removed for examination, they were analyzed for physical changes, cleaned, and compared to a control bullet using a comparison microscope according to standard agency protocols. After the examination, the bullets were replaced in the same locations. Three possible conclusions could be reached during the examination: identification, similarities (inconclusive), or unsuitable. The examination of the bullets involved examining each land impression independently and reaching a conclusion, then analyzing the bullet as a whole to determine if could be identified, despite damage to some land impressions.

Analysis showed that within only a few days of exposure to decomposing tissue, the copper displayed areas of discoloration, which appeared as a marbling effect. The areas of the bullets that were in direct contact with the bone (femur) were consistently lighter in color than the rest of the bullet(s). The copper also underwent oxidation, and patina formed around the exterior of the bullet. During the examinations, the patina was removed to observe the individual marks. The bullets that remained untouched until the conclusion of the study displayed a thicker layer of patina.

Results demonstrate that the number and quality of land impressions still visible after exposure to decomposing human tissue differed according to location, length of exposure time, and contact with bone. The bullets left *in situ* until the end of the data collection period were more heavily corroded than those that were removed and cleaned over time. Over time, the individual characteristics in the land impressions became dulled and less distinct, and pitting became apparent in the copper jackets. Additionally, maggot activity appears to have accelerated the rate of change observed on the bullets, as the bullets placed in Donor 1 (after most of the maggots had left the body to pupate) were identifiable longer than those placed in Donor 2, which progressed through all stages of entomological activity following bullet placement.

This research demonstrates the need to be aware of the potential effects of decomposing tissue on the ability to identify the source weapon that may have fired bullets related to the cause and manner of death of decedents. Additional research is needed to ascertain the effects of different time intervals and possibly those related to the seasons and other climatic factors.

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#### Decomposition, Bullet, Comparison



## Young Forensic Scientists Forum—2020

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### Y4 Recovering Latent Fingerprints From Duct Tape After Removal From Various Surfaces Using Dry Ice

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**Learning Overview:** The goal of this presentation is to provide criminal insight on the most advantageous method for recovering latent fingerprints from duct tape.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing latent print examiners an alternative method of removing duct tape and will assist in determining which latent fingerprint development method works best after applying the dry ice/acetone slush.

Duct tape is a common item of physical evidence found at many crime scenes. Duct tape has been used to facilitate different crimes, and the criminal population utilizes duct tape in a variety of ways. Some victims of violent crime are restrained or bound with duct tape, and duct tape is used to package items related to criminal activity. While there has been research in the area of preserving and recovering latent fingerprints from duct tape, very few studies have examined the use of dry ice for separation from various surfaces. The purpose of this study was to examine how dry ice reduces the adhesive properties of tape, specifically duct tape, and how the removal affects the quality of recovered latent fingerprints.

The researcher in this study examined the quality of recovered fingerprints separated from various surfaces using a dry ice/acetone slush. Initially, a series of preliminary experiments were conducted to observe how specific physical states of dry ice successfully separated tape from wood, metal, and glass. Dry ice was applied in blocks, crushed, powdered, and in an acetone slush. The following brands of duct tape used on each surface included Gorilla® Tape, 3M® Super Tough Duct Tape, 3M® All-Weather Duct Tape, Shurtape®, and 3M® Tough Duct Tape. All of the forenamed duct tapes were separated from wood, metal, and glass. A dry ice/acetone slush (-78.5°C) provided the most successful method for separation.

A latent print development method was used on each of the duct tapes after removal from the surfaces. The various developing methods that were used to recover latent fingerprints included cyanoacrylate fuming followed by Ardrox™ dye, ninhydrin, 1,8-Diaza-Fluorenone (DFO), Wetwop™, and crystal violet. The quality of recovered latent fingerprints was determined by using a grading system based on ridge detail and minutiae points. Preliminary results have shown that cyanoacrylate fuming followed by Ardrox™ dye, Wetwop™, and crystal violet yielded identifiable fingerprints. Additionally, it was observed that the texture of duct tape could interfere with the quality of developed prints, making the ridge detail difficult to see.

The final results of this study will assist latent print examiners by providing an alternative method of removing duct tape and will assist in determining which latent fingerprint development method works best after applying the dry ice/acetone slush.

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#### Latent Fingerprints, Dry Ice/Acetone, Duct Tape





## Young Forensic Scientists Forum—2020

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### Y5 Enhanced Collection and Recovery of Cellular Material, Coupled With Direct Polymerase Chain Reaction (PCR), From Rough Surfaces for Forensic “Touch DNA”

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**Learning Overview:** After attending this presentation, attendees will understand that comparing a novel method of collection of trace DNA samples from smooth to rough surfaces and objects with a novel vacuum device, the optimization and testing of different filter matrices (e.g., polyvinylacrylate vs. polycarbonate), and new high-fidelity polymerases to provide more robust and complete DNA profiles, along with comparing and eventually eliminating DNA extraction methods, serve to train the student in the entirety of the traditional DNA workflow. Ultimately, enhancing collection with a vacuum device and forgoing DNA extraction for direct PCR will create a new workflow for adequately processing trace DNA samples.

**Impact on the Forensic Science Community:** The implementation of the Venturi Vacuum Device (VVD), a simple vacuum technology, into the front-end processes of forensic DNA typing could lead to a reevaluation of standard collection techniques of trace evidence. The VVD and direct PCR will have a positive synergistic effect on downstream processes, providing improved genetic profiles. This device could revolutionize the conventional approach to the collection of challenging forensic samples and the subsequent evaluation of DNA profiles generated and used in criminal casework.

The inclusion of DNA evidence in criminal cases is the gold standard of the contemporary courtroom. Of late, collection of low quantities of cellular material is becoming more common throughout federal and state crime labs that use touch DNA, a class of biological trace evidence that is recovered from the epithelial cells in the sweat and oils on the hands of individuals. However, the challenge remains on how to efficiently collect touch DNA evidence associated with casework ranging from property crimes to sexual assault and murder. The downstream analytical success of such valuable evidence continues to be hampered by inadequacies involved in its collection. The inability of traditional collection matrices—cotton swabs—to adequately pick up, then release cellular material once it has been collected has a direct impact on the success of a useable or full DNA profile. This is especially prevalent with touch DNA of unknown quality and found on rough surfaces where a swab falls apart or cannot easily access a surface to even obtain a sample. Therefore, there is a strong need to enhance the collection of cellular trace evidence from rough surfaces commonly encountered at crime scenes.

This research addresses these inherent collection shortcomings by improving cellular collection and subsequent recovery of DNA, especially from rough surfaces, using a field-portable VVD (patent pending). A VVD creates a vortex of negative pressure by passing compressed air (CO<sub>2</sub>) through a narrow section of piping, generating a powerful suction. Attaching a Polycarbonate Filter (PCF) to the VVD apparatus allows for the use of the vacuum and suction processes to collect the cellular material. The VVD will first be tested on mock touch samples placed on stainless steel bars (smooth surface), then rope and brick (rough surfaces) using a known quantity (100 cells) of epithelial cells placed on the surfaces. Second, true touch samples from 100 individuals handling rough objects will be sampled with the optimized protocol. This will be compared to samples collected with traditional cotton swabs, where cotton swabs tend to rip and tear when used on porous and textured surfaces. Once collection issues have been addressed, the next step is to eliminate loss of DNA in downstream processes due to DNA extraction. The DNA loss resulting from multiple extraction steps and tube changes adversely affect the success of recovering enough DNA to generate a robust profile. With that said, the combination of VVD collection and direct PCR, forgoing extraction and quantitation steps, will significantly increase the probability of obtaining probative DNA profiles.

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#### Touch DNA, Trace Evidence, Forensic DNA Typing



## Young Forensic Scientists Forum—2020

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### Y6 DNA Contamination From Handled Sharpie® Markers Used to Outline Bodily Fluids in a Forensic Laboratory

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**Learning Overview:** After attending this presentation, attendees will better understand contamination via DNA transfer caused in the laboratory and the impact this contamination has on interpreting data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying a method of introducing DNA contamination into the laboratory through common practice and how this can be prevented in order to reduce the likelihood of contamination as much as possible while processing evidence.

With the ever-increasing sensitivity of DNA technology, DNA transfer is more readily detected and observed in fragment analysis data. In a crime laboratory, forensic biologists are handling tools that can instigate this DNA transfer event, leaving traces behind on evidence. This research will determine whether forensic biologists transfer DNA from evidence to evidence using Sharpie® markers that outlined bodily fluids prior to DNA extraction.

Forensic biologists outline bodily fluids with markers before swabbing for further DNA analysis to visualize where the samples are located. This is especially important when the biological material is not visible to the naked eye, such as semen, and an Alternative Light Source (ALS) is required. The marker is used multiple times on separate pieces of evidence, which may or may not be from the same case. This transferring of DNA could contaminate evidence with DNA from separate individuals and from unrelated cases, creating more noise and mixture data interpretation in the fragment analysis results.

A preliminary study was employed to confirm DNA can be extracted from the tips of Sharpie® markers. To do this, human whole blood and human semen were deposited on blue denim and black T-shirt swatches. After the bodily fluid dried for at least one week, the Sharpie® markers were purposely drawn over the bodily fluids, using either a black Sharpie®, metallic silver Sharpie®, or white China Sharpie® marker, depending on the substrate color. The tip of the Sharpie® marker was then swabbed using a Puritan® cotton swab and the double-swab technique. Both a 5% Chelex extraction and a QIAGEN® DNA Investigator Kit were used to determine which extraction method was more efficient. Each sample combination was repeated in quintuplicate with a positive, negative, and substrate control for a total sample size of 128. The samples were quantified using the QuantStudio™ 5 Real-Time Polymerase Chain Reaction (PCR) instrument and the Quantifiler® Human DNA Quantification Kit. The GeneAmp® PCR System 9700 and GlobalFiler® PCR Amplification Kit were used to amplify all samples and a 3130 Genetic Analyzer was used to genotype the samples.

The results obtained from the preliminary study showed DNA can be extracted from Sharpie® markers. This study also showed the QIAGEN® DNA Investigator Kit yielded more efficient results, which were then utilized for further research. After the preliminary experiment, the mock scenario was implemented to re-enact a practical situation in a laboratory. This employed the same techniques; however, the clothing was donated to better simulate a practical situation. Additionally, reference samples were collected via buccal swabs from the individuals who donated the bodily fluid and clothing. The Sharpie® markers were used to outline the bodily fluids on up to three swatches containing biological material from a different source. The samples were extracted, and the data was compared to the reference samples to identify if the Sharpie® markers were transferring DNA in detectable quantities.

Identifying contributing factors to DNA contamination in a crime laboratory is imperative so that measures can be taken to prevent further contamination. This is especially critical for low copy number samples or samples that do not contain enough material for conservation. Recognizing and understanding all possible avenues of contamination will create a more sterile and safer environment for the processing of evidence.

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#### DNA Transfer, Contamination, Fragment Analysis



## Young Forensic Scientists Forum—2020

### Y7 Ultraviolet (UV) Absorption Properties of Synthetic Cathinones

Jane Berger, BS\*, Cedar Crest College, Allentown, PA 18104; Thomas A. Brettell, PhD, Cedar Crest College, Allentown, PA 18104; Matthew R. Wood, PhD, Ocean County Sheriff's Department, Toms River, NJ 08753; Marianne E. Staretz, PhD, Cedar Crest College, Allentown, PA 18104

**Learning Overview:** After attending this presentation, attendees will understand how UV/Visible (UV/Vis) spectrophotometry can be used to determine the wavelength maxima and molar absorptivity of synthetic cathinones.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reporting the UV/Vis spectrophotometric properties of synthetic cathinones, including the wavelength maxima and molar absorptivity in methanol, and how this information can be used to screen seized drugs for these compounds.

Seized drug analyses are routinely performed and are a major part of the workload in crime laboratories across the country. Typically, color or spot tests are used to presumptively identify a controlled substance with subsequent confirmation by Gas Chromatography/Mass Spectrometry (GC/MS). While most traditional drugs of abuse, such as heroin and cocaine, can be presumptively identified by common color tests, this is not the case with synthetic cathinones. The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) has published recommendations for the minimum requirements for the forensic identification of seized drugs.<sup>1</sup> In these recommendations, SWGDRUG has classified analytical techniques incorporated within an analysis scheme into three categories based upon the level of selectivity they achieve. UV/Vis is listed as a category B technique since it is not very selective, particularly when compounds have similar structural properties. For this reason, it has not been a commonly used technique for screening seized drugs. There is very little data reported in the peer-reviewed literature about the UV/Vis spectral properties of synthetic cathinones. For this reason, and the fact that there is limited presumptive test options for these compounds, the UV/Vis spectral properties were investigated. The determination of wavelength maxima and molar absorptivities in methanol were determined for more than 20 synthetic cathinones. Hopefully, this information will be useful to practitioners analyzing seized drugs.

A Beckman Coulter® DU-800 UV/Vis spectrophotometer, Mettler Toledo LabX™ UV/Vis spectrophotometer, and a Cary 3500 UV/Vis spectrophotometer were used to create calibration curves based on dilutions sets prepared using Cerilliant® and Cayman Chemical certified reference standards. Nine working solutions were created from a 10- $\mu\text{g}/\text{mL}$  stock solution of each synthetic cathinone by diluting with American Chemical Society (ACS) -grade spectrophotometric methanol. The concentrations ranged from  $5 \times 10^{-5}\text{M}$  (stock) to  $5 \times 10^{-6}\text{M}$ . A set of stock and working solutions for each synthetic cathinone was prepared in duplicate from separate certified standards with different lot numbers. Spectra were acquired on all three aforementioned instruments for each synthetic cathinone for each dilution set. All spectral data acquired were processed using Microsoft® Excel® for MAC® (version 16.30) to determine molar absorptivity, standard deviation, and Coefficient of Variation percentage (CV%).

Data obtained on all three UV/Vis spectrophotometers were in excellent agreement (CV% < 4%). Wavelength maxima varied slightly for all cathinones depending on the functional group substitution. Methyleneoxy-ring substitution resulted in multiple wavelength maxima. For all cathinones analyzed in methanol, the wavelength maxima were in the range of 230.6nm (methylone) to 279.4nm (methedrone). Regression analysis of plotted data (absorption vs. concentration) resulted in good linear correlation (all above  $R^2=0.99$ ) for every synthetic cathinone analyzed on all three instruments. Molar absorptivity values in methanol ranged from  $1.18 \times 10^5\text{L}\cdot\text{cm}^{-1}\cdot\text{mol}^{-1}$  (methcathinone) to  $1.97 \times 10^5\text{L}\cdot\text{cm}^{-1}\cdot\text{mol}^{-1}$  (methylone).

This presentation reports spectral data for more than 20 synthetic cathinones that have not been reported previously. This includes wavelength maxima and molar absorptivity data recorded from cathinones in methanol. Although UV/Vis spectrophotometry is not highly selective, the information reported here can be used with other analytical data to identify and quantify synthetic cathinones. Sample preparation is simple and the analysis is not time consuming. This study demonstrates that UV/Vis spectrometry has the potential to be used as a screening tool in crime laboratories for the analysis of synthetic cathinones in drug seizures.

#### Reference(s):

1. Scientific Working Group for the Analysis of Seized Drugs Recommendations, Version 8.0, 2019:June 13. [www.swgdrug.org](http://www.swgdrug.org) (last accessed on 31Oct19).

#### Forensic Chemistry, Synthetic Cathinones, UV/Visible Spectrophotometry



## Young Forensic Scientists Forum—2020

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### **Y8 The Possibility of Personal Identification By Measurement of Scapular Volume and Bone Conformation: A Preliminary Assessment With Postmortem Full-Body Computed Tomography (CT)**

*Alissa M. Shida, MS\*, Osaka City University Medical School, Osaka 545-8585, JAPAN; Aoki Yayoi, BA, Osaka City University Medical School, Osaka 545-8585, JAPAN; Kei Ikeda, MD, Osaka City University Medical School, Osaka 545-8585, JAPAN; Naoto Tani, MA, Osaka City University Medical School, Osaka 545-8585, JAPAN; Tomoya Ikeda, PhD, MD, Osaka City University Medical School, Osaka 545-8585, JAPAN; Takaki Ishikawa, MD, PhD, Osaka City University Medical School, Osaka 545-8585, JAPAN*

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**Learning Overview:** After attending this presentation, attendees will learn the value of postmortem imaging for personal identification using scapula morphometric values from postmortem CT.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that with scapular morphometrics, CT data can be used to obtain objective indicators for personal identification.

The value of postmortem imaging for personal identification has clearly emerged in recent years, and applications of postmortem bone imaging to the determinations of sex, height, weight, and other characteristics have been investigated. This study reports on a preliminary investigation into the relationship of scapula morphometric values from postmortem CT to sex, age, height, weight, and other physical properties.

An analysis was performed for 570 adult autopsies (359 men, 211 women; mean age 60 years; range 18–98 years) with no observable injury, damage, or other effects on anatomical structure. CT was performed using a Hitachi Medical Multislice ECLOS-16 system and scapular 3D structure reproduction images were analyzed on a Fujifilm SYNAPSE VINCENT volume analyzer.

Among the various scapular measurement points, this study measured straight-line distance from the most superior point (D) on the superior angle to the most inferior point (C) on the inferior angle and from the most posterior point (I) on the crest of the glenoid fossa border to the spine base center point on the medial border (B), and left-right scapular volume and mean CT values. Sex determination by Receiver Operating Characteristic (ROC) curves of inter-D-C distances in the left and right scapulae showed sensitivity of 0.88–0.91 and specificity of 0.88–0.92, and that by scapular inter-IB distance showed sensitivity of 0.89–0.91 and specificity of 0.89–0.91, indicating high determination capability. Sex determination capability by volume was even higher, at 0.97–0.98 sensitivity and 0.95–0.96 specificity. No strong correlations (no correlation  $\sim r=0.56$ ,  $p<0.001$ ) of scapular measurement values to height or weight were observed. Taken together, the results indicate that with scapular morphometrics, CT data can be used to obtain objective indicators for personal identification.

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### **Computed Tomography, Personal Identification, Scapular Volume**



## Young Forensic Scientists Forum—2020

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### Y9 Testing of a Probe Capture Next-Generation Sequencing Assay for the Analysis of Nuclear Short Tandem Repeat (STR) and Single Nucleotide Polymorphism (SNP) Markers

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**Learning Overview:** The goal of this presentation is for attendees to recognize the value of probe capture enrichment Next Generation Sequencing (NGS) assays in the analysis of genetic markers from single DNA shotgun libraries.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees an alternative method to Polymerase Chain Reaction (PCR) amplification for analyzing challenging samples, including highly degraded samples and mixtures.

DNA from biological samples in forensic casework may be mixed or in degraded condition. In samples with highly degraded DNA, both PCR primer binding sites may not be present on the template DNA fragments, and conventional PCR amplification and Capillary Electrophoresis (CE) analysis of STR may fail. Probe capture enrichment utilizes overlapping biotinylated probes to capture fragmented DNA. Since STR regions cannot directly be targeted for capture, DNA regions flanking the STRs were targeted using probe capture enrichment.

Previously, it was demonstrated that the SNP probe capture/NGS system can capture and sequence DNA fragments as short as 35 base pairs with input as low as 0.5ng while yielding 99%–100% reportable SNP genotypes.<sup>1</sup> Reportability is determined by meeting the minimum threshold requirement for read depth of >500 reads per base. This study tested the performance of SNP (v3.0) and STR (v1.0) probe capture panels with the same shotgun libraries. Three commercial control DNA samples, Promega's® 2800 M, the National Institute of Standards and Technology's SRM2391-c, and Coriell Cell Repositories' NA24149, and 12 blood-derived DNA population samples were prepared using a "shotgun" approach and given unique dual-indexed barcode sequences. DNA shotgun libraries were pooled for probe capture enrichment. The enriched products were sequenced on Illumina® MiSeq®. SNP and STR sequence data were analyzed using NextGENe® and GeneMarker® HTS Software.

Ninety-six percent of SNPs ( $n=496$ ) were reportable using this custom SNP (v3.0) probe capture panel in samples with input amounts of DNA at 25ng. Some of the STR loci could be analyzed by inspection of the sequence read "pile-ups" with reads that spanned the STR corresponding to the known length variants. This "proof of concept" study with this STR (v1.0) panel identified sequence polymorphisms that would be undetectable by conventional CE analysis. The successful development of probe capture NGS for both STR and SNP assays would provide practitioners an alternative method to PCR amplification for analyzing challenging samples, including highly degraded samples and mixtures.

**Reference(s):**

- <sup>1</sup> Bose, Nikhil, Katie Carlberg, George Sensabaugh, Henry Erlich, and Cassandra Calloway. Target Capture Enrichment of Nuclear SNP Markers for Massively Parallel Sequencing of Degraded and Mixed Samples. *Forensic Science International: Genetics* 34 (2018): 186–96.
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### Next Generation Sequencing (NGS), Single Nucleotide Polymorphism, Short Tandem Repeat (STR)



## Young Forensic Scientists Forum—2020

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### Y10 A Profile of Fatal Poisoning Cases Brought for Postmortem Examination at a Tertiary Care Institute in India—A Retrospective Study of 21 Years of Autopsy Cases

*Rajesh Kumar, MBBS, MD\*, Ranchi, Jharkhand 834001, INDIA; Jay N. Pandit, MBBS, MD, All India Institute of Medical Sciences Campus, New Delhi 110049, INDIA*

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**Learning Overview:** After attending this presentation, attendees will understand some principles on trends, magnitude, and profiles of fatal poisoning cases in the national capital region of India.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sensitizing attendees regarding the poisoning problem in India.

Trends of poisoning have been constantly changing throughout the world with the advent of new agents. The high incidence of suicide by poisoning among young adults can be checked by frequent psychological counseling and by tackling their problems sympathetically. Education of the community with regard to proper storage and use will reduce the incidence of poisoning. This presentation will highlight analyzed data with relevant tables and figures.

Poisoning is a significant global public health problem. According to World Health Organization (WHO) data, in 2012, an estimated 193,460 people died worldwide from unintentional poisoning. In 2017, the 55 United States poison control centers provided telephonic guidance for nearly 2.12 million human poison exposures. In India, the National Poisons Information Centre (NPIC) at All India Institute of Medical Sciences (AIIMS) received 16,420 telephone calls for guidance regarding poison exposure between April 2006 and March 2016. Globally, much is known and documented regarding poisoning. Contrarily, in India, less is known regarding poisoning due to the unavailability of published data, access to databases, and very few established national poison surveillance centers for the collection of reliable data.

The incidence of morbidity and mortality due to poisoning in India is among the highest in the world. It is estimated that more than 50,000 people die every year from toxic exposure. Despite the vast number, evidence on prevalence pattern in India is still limited. The goal of this present study was to retrospectively analyze various aspects of poisoning cases conducted at AIIMS, New Delhi, during the period of January 1998 to December 2018. Autopsy reports were studied and data analyzed to discover classes of poisonous agents that have been used in fatal poisoning cases. The data has been analyzed as to the manner of death and frequency of intoxications as well as age and sex of the deceased. A total of 31,848 autopsies were conducted at AIIMS during the past 21 years (1998–2018) with the cause of death as intoxication in 1,118 cases. The spectrum of substances that are abused, taken deliberately, or accidentally is continuously changing, reflecting scientific progress in the pharmaceutical and chemical industry. The present study shows the highest mortality due to agrochemicals poisoning (497). This study clearly demonstrates the uniformity of male preponderance over female in a ratio of 2:1. The most common cause of death in this study was suicidal death (1,016) followed by accidental death (92), and the least were cases due to homicidal death (10). This study shows an overall decrease in the trend of poisoning cases, but control is still needed since the poisons are easily available and less regulated.

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#### Poisoning, Agrochemicals, Suicide



## Young Forensic Scientists Forum—2020

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### Y11 The Detection and Quantification of Trace Fentanyl in Mixtures With a Portable Raman Instrument and Chemometrics

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**Learning Overview:** The goal of this presentation is to describe a detection method based on Surface-Enhanced Raman Spectroscopy (SERS) with a portable Raman instrument and a chemometric model to distinguish mixtures of fentanyl/heroin, fentanyl/cocaine, and fentanyl/heroin/cocaine.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a SERS-based chemometric method for the presumptive screening of opioids. This new method is fast and can rapidly distinguish fentanyl analogs from other drugs.

The abuse of opioids has become a critical issue in the United States over the past five years. New synthetic fentanyl analogs continue to appear in street drugs, resulting in increased threats to the public health. Since the appearance of these new fentanyls, prior screening methods, such as immunoassay, have had difficulty in the detection and analysis of the multiplicity of opioid analogs in the market. This study has been working on an alternative screening method using SERS coupled with metal nanoparticles and aggregating agents. SERS is a rapid screening method that provides molecular fingerprint signals at toxicological concentrations. The procedure is simple and fast, and it is convenient for use in both point-of-care analysis and laboratories. The new method can detect fentanyl analogs, cocaine, and heroin at low to sub ng/mL concentrations, as well as distinguish fentanyls in mixtures with cocaine or heroin, even at levels as low 0.01% fentanyl in the mixture.

The SERS method utilizes gold/silver nanostars in colloidal form that are mixed with magnesium chloride and aggregated. Next, drug samples are added to aggregated silver/gold nanostars and allowed to incubate for five minutes. On the surface of the aggregated nanostars, the creation of hot spots produces localized surface plasmon field effects resulting in an improvement in SERS enhancement. The SERS spectrum provides molecular vibration information that can identify individual compounds. Chemometrics, such as linear discriminant analysis and principle components analysis, were then used to create a model to cluster classes of drug samples, as well as distinguish single drugs and their mixtures. The resultant data assist in the calculation of the percentage of fentanyl in the mixture based on the composite spectra.

The SERS method permits a rapid, easily operated presumptive test for opioids. It is orthogonal to mass spectrometry and sufficiently sensitive to detect compounds at toxicological levels. As a result, it should be particularly useful for the screening of fentanyl analogs, mixtures with heroin and/or cocaine, as well as other novel psychoactive substances and mixtures.

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**Fentanyl Mixtures, SERS, Portable Raman**



## Young Forensic Scientists Forum—2020

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### Y12 Visualizing and Detecting Explosives Through the Use of High-Performance Thin-Layer Chromatography (HPTLC)

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**Learning Overview:** After attending this presentation, attendees will have gained a basic understanding of how to use an HPTLC system to detect explosives.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a novel way to detect explosives in any criminal instance, such as acts of terrorism. This method allows for explosives to be detected and differentiated with the hope of one day being able to trace the components back to an initial source and identify a potential suspect.

As terrorist attacks involving the use of explosives persist around the globe, the development of novel methods for the detection and identification of explosive residues continues to be an important pursuit. Thin Layer Chromatography (TLC) is one of the many analytical methods commonly used to detect explosives. As an extension of the capabilities of TLC, it is proposed that HPTLC in conjunction with chemical development can provide valuable information when attempting to detect explosives.

The goal of this project was to evaluate different mobile phase systems in an attempt to identify which system would provide the best HPTLC separation of explosives. Many different solutions were employed to visualize the explosive solutions such as: a horseradish peroxidase solution, N,N-dimethyl-1,4-phenylenediamine dihydrochloride solution, potassium iodine-starch solution, ammonium thiocyanate-ferrous sulfate solution, ferrous hydroxide solution, modified Griess reagent, 1% diphenylamine solution, 0.02% diphenylbenzidine solution, and 0.2% diphenylbenzidine solution. Out of these nine solutions, 1% diphenylamine in hexane provided the most promising results. The plates were spotted using an automatic sampler. After spotting, the samples were developed in a developing chamber. During development, appropriate solvent phases were investigated to ensure proper separation. To this end, two mobile phases were compared. The first mobile phase tested was an 8:4:3 ratio of toluene, hexanes, and acetone. The second mobile phase explored was an 8:4:3 ratio of toluene, hexanes, and ethyl acetate. After comparison of these two mobile phases, it was determined that a mixture of toluene, hexanes, and ethyl acetate provided the best separation.

Due to the fact that nitroaromatics and nitramine compounds can be detected after irradiating with 254nm Ultraviolet (UV) light; pictures of the plate were taken under white light, 254nm, and 366nm light using a visualizer. The plates were then treated with 1% diphenylamine in hexane by vertically dipping the plates into the solution and allowing them to dry before photographing again under the same conditions. Treating the plates with the 1% diphenylamine solution allows for the identification of nitrite esters. Last, measurements were taken using a scanner, and an overall report of the measurements was generated. At this point in the project; 1,2-dinitrobenzene, 3,4-dinitrotoluene, Ethylene Glycol Dinitrate (EGDN), nitroglycerin, nitroguanidine, Pentaerythritol Tetranitrate (PETN), and picric acid have been examined using this procedure. All the explosives tested, with the exception of nitroguanidine and picric acid, were separated using this method. In the future, this method could be used to detect single-component explosives or explosive mixtures.

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**Forensic Science, Explosives, High-Performance TLC**





## Young Forensic Scientists Forum—2020

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### Y13 The Effects of Storage Conditions and Time on Extracted Ignitable Liquids Using Gas Chromatography/ Mass Spectrometry (GC/MS)

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**Learning Overview:** After attending this presentation, attendees will understand how variables such as temperature and storage containers affect the ability to identify petroleum distillates through GC/MS analysis over several months.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by aiding in the establishment of optimal storage procedures, designed for ignitable liquid extracts, for the preservation of accurate analytical results.

In arson investigations, recovery of an ignitable liquid can be the determining factor in whether a fire is ruled accidental or intentional. In some cases, fire debris recovered from the scene must be archived in the event that a sample must be analyzed at a later time, though little is known about how to properly store extracted samples in order to maintain the integrity of analytical results over time. Up to this point, there has been research conducted comparing analytical results using different storage containers, extraction techniques, and analysis methods over longer periods of time; however, temperature has not yet been considered in conjunction with these other factors. To address this gap in research, the conjoined effect that storage temperature and container have on analytical results over time were tested by storing in two different containers at two different temperatures across a nine-month period.

Ignitable liquids can be classified into eight basic categories and three sub-categories; this study focused on petroleum distillates, just one of these eight classifications, due to their accessibility and low cost. Three petroleum distillates (Crown<sup>®</sup> White Gas Camp Fuel, Crown<sup>®</sup> Paint Thinner, and Crown<sup>®</sup> 1-K Kerosene) classified into different sub-categories (light, medium, and heavy) were selected as samples. Samples were extracted onto activated charcoal strips using a passive headspace method. The charcoal strips were then transferred to a Sirchie<sup>®</sup> Nylon Fire Debris Bag or PTFE-lined glass vial. Once contained, samples were stored under room temperature conditions or refrigerated at 4°C. After storing for intervals of three months, six months, and nine months, extracted samples were subsequently analyzed using GC/MS to visualize peak profiles. The protocol mentioned previously was followed for preparation of “time 0” samples (samples that were extracted and immediately run on the GC/MS with no storage) in order to provide a baseline for comparison purposes. Analysis of sample data was performed through identification of target compounds according to the American Society for Testing and Materials protocol by utilizing the National Institute of Standards and Technology Mass Spectral database.<sup>1</sup> Comparisons were made between all variables (storage time, container, temperature, and among the distillates chosen), noting any loss of target compounds or signal strength. This research may indicate a modification to standard protocol for the storage of extracted ignitable liquids in the future, ensuring accurate results.

**Reference(s):**

- <sup>1</sup> ASTM Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography/Mass Spectrometry. 2014, E1618-14, 1-15.

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**Ignitable Liquid, Arson, Storage Conditions**



## Young Forensic Scientists Forum—2020

### Y14 Testing Kinship Via Mitochondrial DNA on Colony vs. Non-Colony Cats

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**Learning Overview:** The goal of this presentation is to draw attention to a portion of wildlife forensics and examine the social structure of colony and non-colony feral cats (*Felis catus*). Attendees will gain an understanding of Trap-Neuter-Return (TNR) and its role in this study via mitochondrial DNA (mtDNA), the mating structure of feral cat colonies showing degree of relatedness, and the impact of the *Felis catus* population on wildlife forensics.

**Impact on the Forensic Science Community:** This presentation will impact the forensics science community by examining the social structure of feral cats. In addition, *Felis catus* Short Tandem Repeats (STRs) were the first non-human STR evidence used in a court of law.

Populations of feral cats (*Felis catus*) have drawn public attention due to a variety of reasons, including the increasing population size, caterwauling, destruction of property, and decreasing rodent and bird populations. Although controversial, several communities have implemented the TNR method, developed in Rome, Italy.<sup>1</sup> TNR has been praised for population management, but cats are often demonized in wildlife forensics for their tendencies of property destruction and preying on rodent and bird populations. TNR clinics have provided the cat ear tips used in this study, which examined whether colony cats are more likely to be related than cats of the general population (non-colony cats). Colony feral cats are cats that were once domesticated and now returned to the wild, living in a specific environment with other cats. Non-colony feral cats are the same, but do not live in these groupings. Past research shows the importance of mtDNA in examining lineages. The degree of relatedness was to be determined separately for these two groups. The alternative hypothesis for this study is that colony cats are more related than non-colony cats.

Frankie's Friends, a non-profit spay and neuter clinic, was the ear tip provider for this research. The sample size is  $n=40$  colony cat ear tips and  $n=40$  non-colony cat ear tips. The method of extraction employed was the QIAGEN® QIAamp® DNA Mini Tissue Kit. This was followed by a quantitation step using the NanoDrop™ Lite Spectrophotometer by Thermo Scientific™. The average concentration for colony cats was about 11.7ng/uL after extraction. Two primer sequences, Lf15926 and Hf3, were used to amplify the HV1 region of mtDNA and followed up by sequencing with the ABI® BigDye® Kit, 3130 Genetic Analyzer, and Chromas software. The population statistics based on mtDNA testing will be presented.

#### Reference(s):

- <sup>1</sup> Eckert I., Hartl G.B., Markov G., Suchentrunk F. Genetic diversity and integrity of German wildcat (*Felis silvestris*) populations as revealed by microsatellites, allozymes, and mitochondrial DNA sequences. *Science Direct*. 75 (2): 160-174. 2010.

#### Degree of Relatedness, MtDNA, TNR



## Young Forensic Scientists Forum—2020

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### Y15 The Effects of the Evidence Preservation System (EPS) on the Storage of DNA Samples

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**Learning Overview:** After attending this presentation, attendees will be informed about the investigation into the usage of the EPS as a potential viable storage unit in comparison to common laboratory storage practices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by testing a new technology in sample storage that could be utilized in most laboratory settings. This new technology could potentially prove to be the ideal storage environment to help improve and maintain the quality and quantity of DNA.

This research hypothesizes that the EPS unit will have higher quality and quantity DNA samples compared to room temperature, with similar results to 4°C and -20°C storage environments.

Storage of collected samples is a concern for all disciplines of forensic science. Without proper storage, especially DNA samples, the DNA will become unusable as it could become degraded, cross contaminated with other samples, etc. Much of the preliminary research revealed that there were very few studies on the storage of forensic samples regarding ideal protocols, let alone those that involved combining many controlled storage aspects, such as temperature, humidity, and Ultraviolet (UV) exposure. This research focuses on the EPS by Forensic Solutions, Inc., which is a controlled environment able to be programmed to control temperature and humidity while also preventing UV radiation exposure and bacterial growth on samples.

The purpose of this research was to perform comparative studies between normal laboratory storage conditions of -20°C, 4°C, and room temperature environments, and the EPS unit storage by examining their effects on the quantity and quality of degraded DNA samples as well as the drying weight of samples. For the drying weight comparisons, T-shirts were obtained with no specific material preference and were washed to mimic actual use by an individual rather than ideal conditions. One hundred forty-four 2"x2" swatches were then cut from one shirt and weighed before having blood deposited on them. One hundred twenty samples received a blood deposit of 50µL while 24 swatches were left unaltered for negative controls and 24 0.5mL tubes were filled with 50µL of blood to serve as the positive controls. Once dried, these samples were re-weighed, then placed and sealed inside labeled coin packages. These packages were separated into groups of 42 for each of the four environments (30 samples, 6 positive controls, 6 negative controls) and stored in their respective environments for a six-month period.

For the degraded sample comparisons, preliminary testing was done to determine the medium the blood was to be deposited on and the amount of blood that was to be deposited. It was decided that clothing swatches with 50µL of blood would be an adequate medium for this experiment. One hundred sixty-eight 2"x2" clothing swatches were cut from another washed shirt. One hundred forty-four of these samples received a 50µL deposit of blood while the remaining 24 were left unaltered to serve as negative controls. Once dried, 120 of the swatches were then exposed to UV radiation for ten minutes to irradiate the non-deposit side of the swatch for degradation. The samples were then placed and sealed in labeled coin packages. These packages were separated into groups of 42 for each of the four environments (30 samples, 6 positive controls, 6 negative controls) and stored in their respective environments for a six-month period.

Every month, five samples, one positive control, and one negative control were removed from each storage environment from both the degradation study and the drying weight study experiment groups. The degradation samples would undergo extraction, quantitation, amplification, and detection to determine any changes in quality and quantity of the DNA between storage environments. The drying weight samples would have their weights recorded and compared for any weight changes between the storage environments. Due to the importance of the storage of forensic samples across all disciplines, the EPS unit could be a very useful resource that could change and potentially improve the way various forensic samples are stored.

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#### Evidence Preservation System, Forensic Sample Storage, DNA Degradation



## Young Forensic Scientists Forum—2020

### Y16 Generating Artificially Degraded Human DNA in an Environmental Chamber

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**Learning Overview:** After attending this presentation, attendees will better understand the mechanisms of DNA degradation on various substrates when exposed to environmental conditions, such as Ultraviolet (UV), humidity, and temperature over time and their effect on obtaining a full Short Tandem Repeat (STR) profile.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing experimental methods that can be used in preparing artificially degraded DNA over time.

Environmentally damaged samples contain either degraded DNA fragments or contain somewhat intact DNA with single-stranded nicks and DNA lesions that can hinder acquisition of a complete DNA profile. As a result of this, there is a need in forensic research to generate artificial degraded DNA samples that can be used to improve and test STR typing protocols.

Within the field of forensic science, the testing and analysis of DNA has become notably important and contributed in solving crimes over the years. However, the issue on whether or not it is possible to retrieve an accurate DNA profile from degraded blood left behind at a scene is a recurring concern in the criminal justice system. Often, DNA left at crime scenes is scarce and highly degraded due to various environmental exposure conditions, such as heat, humidity, and UV irradiation. Environmentally damaged DNA may result in failure of amplification, and samples may not have a sufficient number of loci, which are needed for match comparisons. Thus, the purpose of this project was to optimize a method to artificially degrade control DNA and blood stains on different substrates (i.e., jeans and cotton shirt) using controlled environmental conditions (i.e., temperature, humidity, UV). This was done to mimic a “casework type” degraded sample that can be later used to: (1) test novel, enhanced methods (e.g., extraction kits, enhanced polymerase or STR kits) for obtaining a complete STR profile; and (2) better understand the mechanisms of degradation on various substrates and conditions.

To accomplish this, either control DNA (e.g., 9947A) or blood was applied to 1" x 1" squares of cotton and jeans fabrics. Next, the fabric squares were placed in the Environmental Chamber (Q-Sun Xe-3 Test Chamber) under conditions to imitate an outdoor environment (i.e., 15 hours of 71% RH, 0.21–0.25 W/m<sup>2</sup>, 35°C, followed by 9 hours of 60% RH, no UV, 35°C) and fabrics were recovered at various time points across 8 days. DNA extraction was performed for the blood samples using the QIAamp<sup>®</sup> DNA Investigator Kit and the EZ1<sup>™</sup> Advanced XL instrument and all samples were quantified using Quantifiler<sup>®</sup> Trio for all of the time points to obtain the DNA concentration (ng/ul) and level of Degradation (DI). STR profiles were obtained using the Identifiler<sup>®</sup> Plus and the THERMO FISHER<sup>™</sup> CE 3130XL to determine the level of degradation (e.g., ski slope effect, locus drop out, and decrease in Relative Fluorescent Units [RFU]) at the various time points.

Analysis Of Variance (ANOVA) and Tukey’s post-hoc statistical analysis revealed evidence of the ski-slope effect (i.e., a decrease in the RFU of larger loci targets compared to smaller ones;  $p=0.001$ ) and a significant decrease ( $p<0.001$ ) in the concentration of DNA recovered from pant samples (avg 5.96±1.70) when compared to shirt samples (avg=8.71±1.95). Furthermore, significantly higher DNA concentrations were recovered from samples at the 72hr time point than samples at 192hr time point ( $p=0.003$ ). However, no significant difference in the degradation index was found for any sample at any time. Overall, results illustrated a promising method that decreased DNA recovery and illustrated a ski-slope effect indicative of typical degraded DNA STR results.

#### DNA Degradation, STR Ski-Slope Effect, Environmental Chamber



## Young Forensic Scientists Forum—2020

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### Y17 Geospatial Analysis of Canadian Drowning Locations

Vienna C. Lam, MA\*, Simon Fraser University, Burnaby, BC V5A 1S6, CANADA; Barbara Byers, HBA, Lifesaving Society, North York, ON M2J 1P8, CANADA; Gail S. Anderson, PhD, Simon Fraser University, Burnaby, BC V5A 1S6, CANADA

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**Learning Overview:** After attending this presentation, attendees will better understand how geospatial tools can be applied to identify environmental risk factors associated with fatal incident locations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how new geospatial techniques can be used to enhance current practices in risk mitigation of aquatic landscapes.

This presentation will assess coroner and medical examiner data of fatal drowning accident propensity and Emergency Medical Services (EMS) access in Canadian waters from January 2006–December 2016 (an 11-year period). The Response Time (RT) between when a call is made to EMS and EMS arrival is known to be a strong indicator of patient survivability. Studies have shown that when the initial call cannot be made or there are lengthy delays that result in an RT past four to six minutes, chances of favorable neurological outcomes diminish dramatically.<sup>1</sup> It is for this reason that stable cellular reception plays an important role in fatal drowning risk reduction and for the purpose of contacting EMS when accidents near and within water take place. In Canada, there are many attractive, pristine lakes without commuter road networks that can be readily accessed, yet many of these areas do not have cellular coverage. The aim of this study was to identify the strength and type of cellular network available at Canadian drowning locations using geospatial analysis tools as a means of identifying areas of heightened risk due to unstable or limited cellular reception.

In this study, the geospatial coordinates of drowning locations were collected by the Lifesaving Society and based on coroner and medical examiner files from across Canada. Case demographics were assessed to identify risk factors associated with individual characteristics and environmental, geographic features. The spatial perimeters of lake systems and ocean fronts were then identified in this study using the Abacus Dataverse Network in the form of Canadian maps produced by DMTI Spatial. Cell towers were then identified for all locations, along with information on the number of cells per tower and the approximate range and strength of the signal based on ideal weather conditions through Statistics Canada. The network type and generation of broadband cellular network technology, such as Global System for Mobile Communications (GSM), Code Division Multiple Access (CDMA), Universal Mobile Telecommunications System (UMTS), and Long Term Evolution (LTE), was also used to analyze the type of coverage available. Results show that a hierarchical processing of identified fatal drowning locations can successfully be categorized through an assessment of EMS access of these locations. There are certain lakes that pose a higher risk because of limited cell reception, and their risk can be categorized based on the number of reported incidents, as well as the type and amount of reception that exists at each site.

#### Reference(s):

1. Ono, Y., Hayakawa, M., Iijima, H., Maekawa, K., Kodate, A., Sadamoto, Y., Mizugaki, A., Murakami, H., Katabami, K., Sawamura, A., Gando, S. (2016). The response time threshold for predicting favourable neurological outcomes in patients with bystander-witnessed out-of-hospital cardiac arrest. *Resuscitation*, 107, p. 65-70.

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#### Risk Assessment, Drowning Fatalities, GIS



## Young Forensic Scientists Forum—2020

### Y18 A Modern Trail of Tears: The Missing and Murdered Indigenous Women (MMIW) Crisis in the United States

A. Skylar Joseph, MS\*, Elbow Lake, MN 56531

**Learning Overview:** After attending this presentation, attendees will understand the history leading up to the current MMIW crisis; top risk factors for MMIW; why this crisis needs to be addressed at both the local and national levels; and preliminary actions that can be taken by local law enforcement agencies and forensic science divisions to help mitigate this national crisis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the ongoing crisis of MMIW that is currently being experienced in cities across the United States and Canada and will offer some preliminary actions that forensic science practitioners can implement in order to facilitate positive change regarding this issue.

In the United States, American Indian and Alaskan Native people experience higher rates of violence than all other ethnicities. On some reservations, indigenous people experience murder at a rate of ten times the national average.<sup>1</sup> Additionally, homicide is the third-leading cause of death for indigenous women and girls between the ages of 10–24 years and the fifth-leading cause of death for indigenous women between 25–34 years of age.<sup>1</sup> The purpose of the present research has been to investigate the MMIW crisis, determine the cities with the highest rates of MMIW, and to offer suggestions to help fix the root causes for this national crisis.

In the present study, missing persons data was analyzed from the National Missing and Unidentified Persons System (NamUs), the National Crime Information Center (NCIC), and from data collected and published in a report produced by the Urban Indian Health Institute (UIHI).<sup>2</sup> Additionally, unidentified persons data was analyzed from NamUs, and data regarding murder cases of indigenous women was analyzed from the UIHI report.<sup>2</sup> Locations with the highest rates of MMIW cases in the country were identified as potential “hot spots.” Additional analysis of these locations took into account demographic data from the United States census, fracking locations in the United States, and other socio-political factors that could be affecting the MMIW case prevalence in these areas.

Following the analysis, a total of 23 locations were determined to be “hot spots” for MMIW cases. Of these 23 locations, five were identified as being of the highest priority for intervention due to their disproportionately high rates of MMIW cases. These locations are Albuquerque, NM; Anchorage, AK; Gallup/McKinley County, NM; Seattle/King County, WA; and Tacoma/Pierce County, WA. Furthermore, hydraulic fracking across the United States seems likely to be a contributing factor in the rate of MMIW cases in 9 to 16 of the identified “hot spots.”

Currently in the United States, despite a national movement to raise awareness to the MMIW crisis, there has yet to be any actions taken by the federal government to address this issue. The present study has served to identify the primary “hot spots” for MMIW cases and has isolated some of the key contributing factors to this national problem. With this information, a more direct, community-based action plan can be developed in each of these “hot spots” to provide more immediate help to these communities. In order to most holistically address this issue, actions need to be taken at both the local and federal levels, and this study aims to highlight what those initial actions should be.

#### Reference(s):

- <sup>1</sup> Savanna’s Act, H.R. 2733, 116th Cong., 1st Sess. (2019).
- <sup>2</sup> Lucchesi, A., and Echo-Hawk, A. (2018). *Missing the Murdered Indigenous Women & Girls: A Snapshot of Data from 71 Urban Cities in the United States*. Seattle: Urban Indian Health Institute. Retrieved from <http://www.uihi.org/wp-content/uploads/2018/11/Missing-and-Murdered-Indigenous-Women-and-Girls-Report.pdf>.

#### Indigenous Women, Racial Violence, Missing Persons



## Young Forensic Scientists Forum—2020

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### Y19 A Longitudinal Study of the Effects of Storage Conditions on DNA Recovery From Condoms

*Claire J. Loretta\**, Pittsburgh, PA 15203; *Lisa R. Ludvico, PhD*, Duquesne University, Pittsburgh, PA 15282; *Pamela L. Marshall, PhD*, Duquesne University, Pittsburgh, PA 15282; *Stephanie J. Wetzel, PhD*, Duquesne University, Pittsburgh, PA 15282

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**Learning Overview:** After attending this presentation, attendees will understand the relationship between storage duration, storage temperature, and condom brand on the quantity and quality of male DNA recovered from condoms.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a simple, yet essential, proof-of-concept that has yet to be investigated—the impact of condoms on DNA degradation. This presentation will serve as the framework for future studies so that the individual variables in question can be studied in greater detail.

With increased public awareness of the value of DNA in criminal investigations, the number of sexual assaults involving condom use has also increased. Maximizing the utility of evidence—both biologically and chemically—has presented a variety of challenges to an assortment of forensic professionals, such as crime scene investigators, detectives, and laboratory technicians.

Typically, the most crucial evidence in a sexual assault investigation is collected from the survivor during a medical forensic examination in the form of a Sexual Assault Kit (SAK). Unfortunately, the backlog of untested SAKs in the United States is immense. However, condoms are not collected with SAKs and therefore are separately submitted for forensic testing. Because condoms circumvent the SAK backlog, they are often processed much more rapidly, creating a huge advantage for investigators that may allow them to identify a perpetrator without SAK evidence.

While the consequences of DNA degradation are well established across a wide variety of conditions, literature pertaining to the influence of condoms on DNA degradation is practically non-existent. Of the literature concerning condoms, research is primarily focused on the detection and identification of chemical compounds found within condom lubricants. The majority of these efforts contribute to a broader objective—to establish a universal classification scheme and to construct a database of sexual lubricant profiles. Though sexual lubricant analysis is an important and rapidly growing field of trace evidence, it does not exhibit the same discriminatory power and identification capacity that DNA does.

The objective of this study was to investigate the effects of condoms on DNA degradation; it established a fundamental understanding of the relationship between DNA, condoms, and storage conditions.

Both DNA quantity and quality were measured across three variables: (1) time, (2) temperature, and (3) condom brand. Neat semen from one male was aliquoted into condoms from three manufacturers (Trojan<sup>®</sup>, Durex<sup>®</sup>, and Sustain<sup>®</sup>), followed by sample storage at three different temperatures (25°C, 4°C, and -20°C). The total storage duration was one year (52 weeks) with sample analysis at four separate intervals:  $t_0=0$  weeks,  $t_1=16$  weeks,  $t_2=32$  weeks, and  $t_3=52$  weeks.

All samples were extracted using the DNA IQ<sup>™</sup> System and quantified via a QuantStudio<sup>™</sup> 5 Real-Time PCR System using the Quantifiler<sup>®</sup> Human DNA Quantification Kit. The sample extracts were then amplified with the GlobalFiler<sup>™</sup> PCR Amplification Kit via a GeneAmp<sup>®</sup> PCR System 9700; all samples underwent capillary electrophoresis in a 3130 Genetic Analyzer and were analyzed with GeneMarker<sup>®</sup> HID software. In addition to DNA analysis, microscope slide smears were prepared and stained with Christmas Tree stain to evaluate sperm morphology. Finally, the primary chemical compounds of each condom lubricant were identified using quadrupole Time-Of-Flight Liquid Chromatography/Mass Spectrometry (qTOF-LC/MS) performed on an Agilent<sup>®</sup> 6530 qTOF-LC/MS.

The quantities of DNA at  $t_1$  and  $t_2$  were relatively consistent. All samples stored at 25°C had DNA concentrations of 0.01ng/μL or less, while samples stored at 4°C and -20°C had concentrations between 2.35–3.78ng/μL at  $t_1$  and 2.25–8.56ng/μL at  $t_2$ . No significant differences in DNA concentrations were observed between the three condom brands or between the storage temperatures of 4°C and -20°C.

By investigating the progression of DNA degradation within condoms, consistent methods for optimal condom preservation can be employed. Furthermore, supplementing a database of the chemical profiles of sexual lubricants with biological counterparts would considerably increase the value and versatility of such a database.

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#### Condoms, DNA Degradation, Storage



## Young Forensic Scientists Forum—2020

### Y20 The Benefits of Automation in Forensic Toxicology: A Lean Six Sigma and Cost-Analysis Approach

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**Learning Overview:** After attending this presentation, attendees will be proficient in Lean Six Sigma and cost-analysis techniques used to identify inefficient methods and processes in the laboratory.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the advantages of automating extraction and preparation methods for toxicological analysis.

**Background:** Reducing backlogs, improving turnaround times, and streamlining methodology are major focus points for forensic toxicology laboratories. A factory-minded approach in which cases are considered widgets will allow a laboratory to identify areas for improvement. Lean Six Sigma is a process that relies on a collaborative team effort to improve performance by systematically removing waste and reducing variation. By applying this approach combined with a detailed cost-analysis, inefficient or costly processes in the laboratory can be identified. Analytical methods identified in this exercise are excellent candidates for automation transition. Fully automated instruments and equipment, such as the Randox<sup>®</sup> Evidence Analyzer and Hamilton<sup>®</sup> Starlet, are excellent options for automation.

**Method:** Each standard operating procedure used by the Alabama Department of Forensic Sciences was reviewed and observed. An itemized list of consumables was created, and the cost of each item was obtained from previous purchase orders. The average analyst time, salary, and hourly wage were calculated and used to determine the cost of analyst time dedicated to each assay. An MS<sup>®</sup> Excel<sup>®</sup> template for cost compilation (with and without personnel cost) was created in order to determine the cost of each individual method. The cost per maximum-sized batch and cost per case of each assay was calculated. Cost comparison between assays are listed as follows: (1) blood drug screening methods using the Tecan Evo<sup>®</sup> 75 (semi-automated) versus Randox<sup>®</sup> Evidence Analyzer (fully automated), (2) oral fluid drug confirmation methods using Dispersive Pipette Extraction (DPX) on the INTEGRA VIAFLO 96 (semi-automated) versus Hamilton<sup>®</sup> Starlet (fully automated), and (3) opioid quantitation methods using traditional Solid Phase Extractions (SPE) (manual) versus Thermo Scientific<sup>™</sup> SOLA $\mu$ <sup>™</sup> SPE well plate on Hamilton<sup>®</sup> Starlet (fully automated).

**Results:** The most costly and inefficient method identified was the opioid blood quantitation method by traditional SPE. When transitioned to the fully automated Hamilton<sup>®</sup> Starlet, the cost per case and extraction time were reduced by 59% and 50%, respectively. In addition, the sample capacity (batch size) was doubled compared to traditional SPE. Similarly, the oral fluid drug confirmation method using the Hamilton<sup>®</sup> Starlet reduced the cost per case and extraction time by 29% and 38%, respectively. The sample capacity was doubled compared to the semi-automated confirmation method with the INTEGRA VIAFLO 96. The cost per case of a blood drug screen with the fully automated Randox<sup>®</sup> costs 10% more than a blood drug screen with the semi-automated Tecan. However, Randox<sup>®</sup> requires 64% less analyst time per batch.

**Conclusion:** Lean Six Sigma entails removing waste, increasing speed, and reducing variation. A major component of time is the sample preparation and extraction prior to instrumental analysis. If this can be automated, a scientist can focus on other aspects of their duties, such as reporting, reviewing, or providing expert witness testimony. This study provides a better understanding of cost per case, cost per batch, and major contributors to cost, including personnel time. Suggested strategies to reduce inefficiencies and costs include maximizing batch size and reducing analyst time by converting methods to full automation. Future studies will involve evaluating instrument and data analysis cost and time.

#### Cost Analysis, Automation, Lean Six Sigma





## Young Forensic Scientists Forum—2020

### Y21 Further Development of Scoring Rules for Sample Comparisons Using Automated Particle Micromorphometry of Aluminum (Al) Powders

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**Learning Overview:** After attending this presentation, attendees will better understand various strategies currently being researched for the interpretation and discrimination of forensic explosive evidence associated with Al powders. Attendees will also gain an understanding of how automated microscopical imaging, in combination with various statistical methods, may aid in forensic investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the application of Al particle micromorphometry as a quantitative method for the characterization and comparison of explosive evidence, which may also provide valuable lead information for forensic investigations.

**Hypothesis Statement:** The use of automated microscopy in combination with various processing and statistical techniques will provide further source discrimination and investigative leads in source identification involving the use of Al powders in Improvised Explosive Devices (IEDs).

IEDs are often composed of commercial or readily available materials. One such material that can be obtained from multiple sources is Al powder; a common metallic fuel, it has many legitimate uses and applications, making it an easily accessible material. Although many of these sources are marketed in powder form, sources not already in powder form can be easily and inexpensively produced via simple methods found online. This circulation of amateur methodologies for the production and usage of Al powder has put dangerous and destructive tools in the hands of homemade bomb makers, creating the need for new and quantitative identification and analysis methods. For this reason, it has become increasingly important to evaluate not only the composition of such devices, but also to analyze and distinguish between various forms of Al powder for additional investigative and intelligence value.

Previous work done on this research project has displayed the ability to differentiate between methods of Al powder manufacturing (i.e., industrial vs. homemade) through the use of various microscopy techniques: automated particle micromorphometric analysis as well as Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (SEM/EDS). These techniques have shown differentiation in morphology and surface characteristics among these powders. Further, the comparison of similarities and dissimilarities has been statistically scored and evaluated to determine the viability of the method to distinguish between two samples/sources based on 17 characteristics. This presentation aims to build upon previously presented work: the analysis of more Al powder samples has allowed for a more robust data set, and additional statistical approaches have been developed and tested on these high-dimensional, large data sets.

In this work, slides intended for automated imaging were prepped using ~1,000 $\mu$ g of bulk Al powder placed into a microtube containing Permout<sup>®</sup> mounting medium, then mixed thoroughly to avoid uneven dispersion before a set aliquot (via pipet) was placed dropwise onto a slide with a coverslip firmly set over it. This process was conducted such that for each sample, there were a total of seven subsamples (microtubes) and three aliquots (taken from the same microtube), for a total of 21 coverslips per sample. Once mounted, the Al samples were imaged using a transmitted light microscope fitted with an automated stage and automated z-focus. For each coverslip, 200 images were taken at random Fields Of View (FOV) to statistically characterize the distribution of particles in a subsample. Once imaging was complete for each subsample, the resulting data was batch processed using a commercial image analysis software with customized code to fit the parameters to be measured; the image was converted to a binary image, and particles were then measured and counted for each field of view. Seventeen parameters were measured, accounting for various size and shape characteristics of the particles.

Using various statistical means (various machine learning methods applied to summary statistics of subsamples and score-based methods for measuring the dissimilarity of distributions of particles), empirical tests have thus far shown that the ability of the proposed method to correctly characterize the type of powder based upon the 17 parameters is significant. Further, it has displayed that in most instances of incorrect or misidentification for data attribution to a certain sample, the sample has been misidentified with another sample of the same type (e.g., foil sample A misattributed to foil sample C). Both within-source and between-source score distributions were analyzed statistically to further determine how to improve upon the method; various trials utilizing different portions of data were implemented to determine which factors affected results and how those findings may allow for improvement of the analytical and interpretative methods.

#### Improvised Explosive Devices, Aluminum Powder, Micromorphometric Analysis



## Young Forensic Scientists Forum—2020

### Y22 Gas Chromatography With Dual Cold Electron Ionization Mass Spectrometric and Vacuum Ultraviolet Detection (GC/MS-VUV) for the Analysis of Phenylethylamine Analogs

Jordan L. Tanen\*, Rutland, VT 05701; Ioan Marginean, PhD, The George Washington University, Washington, DC 20007; Ira S. Lurie, PhD, The George Washington University, Washington, DC 20007

**Learning Overview:** After attending this presentation, attendees will understand the advantage of GC/MS-VUV in improving confidence in the analysis of Phenylethylamine (PEA) analogs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a rapid method for the identification of PEA analogs involving no liquid-liquid extraction and/or derivatization. This study provides a novel set of tools for the reliable identification of emerging drugs.

PEAs belong to a class of psychoactive drugs that have become more prevalent during the past several years. Certain PEAs were popularized by Alexander Shulgin in his book *PIHKAL*.<sup>1</sup> Many of these drugs are currently being synthesized to circumnavigate drug laws. Online availability results in easy access to many emerging drugs. There have been several deaths attributed to 2C PEAs in both the United States and Europe. Several case studies have shown the adverse effects of PEAs in humans. Because of these reasons, drugs like synthetic PEAs are controlled substances scheduled by the Drug Enforcement Administration.

The majority of drug analyses are commonly performed by GC with Flame Ionization Detection (FID) or MS detection. MS detection is preferable due to its ability to identify compounds based on mass-to-charge ratios and fragmentation patterns, whereas identification by FID relies heavily on the retention time of analytes. The analysis of PEA analogs, including positional isomers, with classical detection methods such as MS poses a few challenges for the forensic chemist. Classical electron ionization of certain compounds, such as phenethylamine analogs results in mass spectra with little to no molecular ions. The mass spectra are also insufficient for discrimination of certain positional isomers. This study demonstrates that the use of GC/MS-VUV can improve the analysis of PEAs. Cold electron ionization can increase the relative intensity of the molecular ions in mass spectra, as well as providing major fragments, while VUV can discriminate between most positional isomers.

Excellent chromatographic performance was obtained for PEA analogs by adding sodium bicarbonate to methanolic solutions of standard compounds. From a mixture of 40 PEA compounds, 21 were resolved with a resolution greater than 1 using VUV detection. However, all solutes could be resolved using a combination of MS detection (single ion monitoring) and/or VUV detection (deconvolution). The relative intensity of the molecular ion was increased for all 2C compounds, while molecules in the NBOMe class showed lower or no increase in molecular ion relative intensity. VUV spectra can be added to a library database as an aid for the identification of PEAs, including positional isomers. The VUV software is also capable of deconvoluting coeluting analytes. PCA was performed on both VUV and MS data of 5 dimethoxymamphetamine positional isomers to demonstrate the improved capability for discrimination of VUV detection.

#### Reference(s):

<sup>1</sup>. A.O. Shulgin et al. 1991. *Pihkal: A Chemical Love Story*. Transform Press.

#### Substituted Phenylethylamines, Cold Electron Ionization, Vacuum Ultraviolet Detection



## Young Forensic Scientists Forum—2020

### Y23 Improved Methods for the Genetic Identification of Burned Skeletal Remains

Kadir Dastan, PhD\*, Istanbul Yeni Yuzyil University, Istanbul 34010, TURKEY; Emel Hulya Yukseloglu, PhD, Istanbul University-Cerrahpasa, Istanbul 34010, TURKEY

**Learning Overview:** The goal of this presentation is to offer best-practice procedures for the extraction of DNA from human cremated remains and to recover DNA useful for identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a predictive model to determine which cremated human remains are likely to produce the best results for the extraction of DNA.

The cremation process consists of burning the corpse at temperatures of up to 1,000°C, reducing it to bone remains or bone fragments, which are often ground to a consistency similar to that of sand. In the identification of forensic cases, it is critical to obtain information on the victim's identity from burned bone and teeth remains.<sup>1</sup> When bone and teeth are burned, both their physical and chemical properties change significantly, which prevents anthropological evaluations and successful DNA profiling. Heat causes difficulties in the identification techniques in which bones and teeth are used, depending on the degree of temperature exposure. Therefore, it is necessary to evaluate the results of observation and analysis, and the information obtained in a certain order, with the changes occurring in bone and teeth during the burning period. The identification of burned or buried bones and teeth has always been a difficult process due to the diminished quality and quantity of DNA, and it is still a challenge.<sup>2</sup> Due to heterogeneity in DNA due to the heterogeneity within the bone, unfortunately, the only reliable method for obtaining DNA from cremated samples such as burned bones and teeth is still not available in the literature.<sup>3</sup>

Previous research on DNA analysis of cremated remains has shown limited success.<sup>4</sup> This study examines adjustments or improvements throughout the DNA analysis workflow to improve methods of identifying human cremated remains. DNA was extracted from bone fragments using a commercial silica-based method or an enhanced extraction method that modifies the commercial kit to determine which process resulted in maximum DNA recovery. Several extracts from a single bone fragment were combined, then concentrated with an Eppendorf® Vacufuge. All extracts were quantified using the real-time Polymerase Chain Reaction (PCR) to evaluate the recovery of nuclear or mitochondrial DNA. Concentrated extracts were amplified using the GlobalFiler® PCR amplification kit with an increased PCR cycle protocol and analyzed using capillary electrophoresis or a complete mitochondrial DNA genome multiplex developed internally for sequencing in the Illumina® MiSeq®. Multiple samples from individual sets of remnants were examined to assess the consistency of the results. The results indicate that low levels of nuclear DNA can be recovered from the cremated bone with partial STR profiles obtained from combined and concentrated extracts; however, exaggerated stochastic effects, such as increased stuttering, allele fall, and peak height imbalance, were observed in some profiles due to the low amount of initial template and the use of an increased cycle PCR, thus complicating the interpretation of the profile.

#### Reference(s):

1. Gamba, Cristina et al. Genome flux and stasis in a five millennium transect of European prehistory. *Nature Communications* 5 (2014): 5257.
2. Godinho, Ricardo Miguel, D. Gonçalves, and A.C. Valera. The pre-burning condition of Chalcolithic cremated human remains from the Perdigoões enclosures (Portugal). *International Journal of Osteoarchaeology* (2019).
3. Johnson, Rebecca N., Linzi Wilson-Wilde, and Adrian Linacre. Current and future directions of DNA in wildlife forensic science. *Forensic Science International: Genetics* 10 (2014): 1-11.
4. Samsuwan, Jarunya et al. A method for extracting DNA from hard tissues for use in forensic identification. *Biomedical Reports*. 9.5 (2018): 433-438.

#### Cremated Bone, Burned Teeth, Forensic Identification



## Young Forensic Scientists Forum—2020

### Y24 The Efficiency of DNA Isolation and Profiling From Burned Human Teeth Remains

Kadir Dastan, PhD\*, Istanbul Yeni Yuzyil University, Istanbul 34010, TURKEY; Emel Hulya Yukseloglu, PhD, Istanbul University-Cerrahpasa, Istanbul 34010, TURKEY

**Learning Overview:** The goal of this presentation is to offer best-practice procedures for the isolation of DNA from burned human teeth remains and to recover DNA useful for identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a predictive model to determine which incinerated human teeth remains are likely to produce the best results for Short Tandem Repeat (STR) analysis.

Because teeth are the hardest tissue in the human body and one of the most abundant types of biological remains available in forensic cases, this study focused on evaluating the effectiveness of DNA isolation from burned teeth and obtaining a DNA profile under different conditions of exposure to temperature and time.<sup>1,2</sup>

Twenty-five healthy erupted third molars, aged 18 to 71 years, were collected. The teeth were divided into seven groups and treated at different temperatures: 100°C, 200°C, 300°C, 400°C, 500°C, 600°C, and 700°C. The teeth of each group were treated at the assigned temperature for 1 minute, 5 minutes, 10 minutes, and 15 minutes, extracting one tooth after each period of time. The control and burned teeth were mechanically ground and subjected to DNA extraction and amplification. Based on the amplification data, it was not possible to obtain DNA from the teeth subjected to 400°C for 10 and 15 minutes, 500°C for 15 minutes, 600°C for 5 minutes, and 700°C for 5, 10, and 15 minutes. In the first temperatures and times, 100°C and 200°C, 1 and 5 minutes, it was possible to obtain an amplification similar to that of the controls; however, in most STRs, the amplification was very low from 300°C for 1 or 5 minutes onward.

This indicates that even in teeth burned and subjected to high temperatures, it is possible to amplify the DNA, at least the domestic DNA; however, the data also show that it would be difficult to obtain an STR profile, probably due to the size of these regions that makes them more prone to degradation.<sup>3</sup> The results of this research provide a quantitative study for obtaining a DNA profile of burned teeth.<sup>4</sup> Future research could extend these results by analyzing other potential regions of DNA for identification under the same conditions.

#### Reference(s):

1. Samsuwan, Jarunya et al. A method for extracting DNA from hard tissues for use in forensic identification. *Biomedical reports* 9.5 (2018): 433-438.
2. Godinho, Ricardo Miguel, D. Gonçalves, and A.C. Valera. The pre-burning condition of Chalcolithic cremated human remains from the Perdigões enclosures (Portugal). *International Journal of Osteoarchaeology* (2019).
3. Johnson, Rebecca N., Linzi Wilson-Wilde, and Adrian Linacre. Current and future directions of DNA in wildlife forensic science. *Forensic Science International: Genetics* 10 (2014): 1-11.
4. Gamba, Cristina et al. Genome flux and stasis in a five millennium transect of European prehistory. *Nature Communications* 5 (2014): 5257.

#### Burned Teeth, DNA Isolation and Profiling, Forensic Identification



## Young Forensic Scientists Forum—2020

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### **Y25 An Investigation of DNA Methylation Markers Indicative of Body Mass Index (BMI)**

*Nicole Fernandez Tejero, BS\*, Ponce, PR 00732; Quentin T. Gauthier, MSFS, Florida International University, Miami, FL 33172; Bruce R. McCord, PhD, Florida International University, Miami, FL 33199*

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**WITHDRAWN**



## Young Forensic Scientists Forum—2020

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### Y26 Cost-Effective Robust Authentication and Environmental Monitoring of Forensic Evidence

*Peter Gompper\*, San Francisco, CA*

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**Learning Overview:** After attending this presentation, attendees will have learned about the weaknesses of existing forensic evidence containers as they relate to the detection of lost, counterfeit, or degraded material. Attendees will also have learned about the utility of the “last mile” problem when developing solutions to these problems, and why custody-chain tracking is unable to support solutions to such events. Finally, attendees will have learned about recent innovations in packaging and how they support reliability and efficient evidence processing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing and elaborating on the necessity of secure unit-level evidence packaging as the optimal means of meeting guidelines. This presentation will also propose performance actions, including how best to: (1) detect an incursion across the surface boundary of an evidence package; (2) authenticate evidence using encryption; (3) register the environmental status of biological evidence; (4) support custody chain requirements (i.e., data regarding location, date, responsible contact, etc.); and (5) support inspection readiness efforts and performance initiatives for agencies unequipped with advanced evidence management systems.

From crime scene to presentation at court, forensic material evidence is at risk of careless handling, theft, counterfeiting, tampering, and environmental degradation. Such “triggering events” can become liability issues for law enforcement agencies, crime labs, courts, and communities dependent upon the credibility of criminal justice systems. Property and evidence managers attempt to mitigate against a wide variety of events by manual and semi-automated processes that focus on custody chain monitoring, tracking in-transit evidence, and controlling access to evidence rooms. The weakness of both manual and semi-automated systems is the lack of unit-level detection of an event. For instance, containers with unique identifiers—such as signature lines, barcodes, or adhered Radio Frequency Identification Devices (RFID) tags—can easily be broken, copied, replaced, or decrypted to thwart systems-level security. Without robust unit-level security, systems-level weaknesses leave evidence at risk, at any point along the custody chain.

To reliably and conveniently authenticate evidence without additional cost or burden to evidence handlers, an electronic Tamper-Evident Packaging (eTEP) was developed. The packaging acts as a one-time irreversible surface monitor capable of communicating to smart phones, electronic devices, and evidence management systems in the submission, handling, presentation, and disposition of items of evidence. Additionally, to support best practice in the maintenance and integrity of biological evidence, the eTEP laminate was designed to detect environmental changes (as outlined by the National Institute of Justice/National Institute of Standards and Technology (NIJ/NIST) Technical Working Group on Biological Evidence, NIST Interagency/Internal Report (NISTIR) 7928).

As a part of the National Science Foundation’s I-Corps program, this presentation will outline the innovation to conference attendees and request feedback and insight regarding potential usability within varying agencies.

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**Evidence, Monitoring, Theft**



## Young Forensic Scientists Forum—2020

### Y27 The Evaluation of DNA Extraction Methods for Chewing Gum Samples

Chelsea Jones\*, Sharpsburg, GA 30277; Ashton B. Jones\*, Fuquay-Varina, NC 27526; Britannia J. Bintz, MSc, Cullowhee, NC 28723; Frankie L. West, PhD, Cullowhee, NC 28723

**Learning Overview:** After attending this presentation, attendees will better understand three different DNA extraction methods, including the PrepFiler® BTA™ method, Phenol-Chloroform-Isoamyl Alcohol (PCIA) purification, and a combined protocol. A comparison of the extraction yields, degree of DNA degradation, sample purity, and the advantages and disadvantages of each method will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by ultimately enabling analysts to make informed decisions regarding the selection of the most appropriate DNA extraction method for chewing gum samples.

Chewing gum samples are potential sources of DNA in forensic casework. Historically, several different methods have been used to extract DNA from chewing gum samples, however, few systematic studies have been conducted to show whether one method is superior to others. In this work, two commonly used methods were tested, including the Applied Biosystems® PrepFiler® BTA™ kit and the PCIA purification method. The PrepFiler® BTA™ kit is often employed in crime laboratories because it is efficient and designed for problematic sample matrices, including bones, teeth, and adhesives. The PCIA method, however, is not as commonly used today because it is labor intensive and requires the use of toxic organic solvents. Per research, no study has been conducted to show that the PrepFiler® BTA™ method results in higher DNA yields than PCIA purification for chewing gum samples. In addition, an optimized protocol was also tested, which included sample lysis using the PrepFiler® BTA™ buffer followed by DNA purification with PCIA and PrepFiler® BTA™ magnetic beads.

It was initially hypothesized that the optimized protocol would be superior because: (1) it would lead to a high yield given that peer-reviewed literature suggested that the use of PCIA results in complete digestion of the chewing gum sample, leading to the release of all DNA present within the gum; and (2) that the extract would be free of inhibitors and other contaminants since multiple purification steps were included. Each method was tested independently by extracting DNA from forensically relevant chewing gum samples. To create these mock samples, gum was chewed for a period of five minutes by a male donor and was then buried outdoors in soil for one week. During this time, environmental humidity and temperature were monitored. DNA was then extracted from the samples, using one of the three protocols described above. The extracts were then assessed for yield, degree of degradation, and presence of inhibitors using quantitative Polymerase Chain Reaction (qPCR) and the Applied Biosystems® Quantifiler® Trio kit. Results were then compared to determine which method, if any, was most efficient at producing large quantities of high-purity, high-molecular weight DNA. DNA yields obtained from qPCR were normalized, then statistically compared using Analysis of Variance (ANOVA). This analysis showed that PCIA purification alone produced statistically significantly higher yields of DNA than the other two extraction protocols tested.

After these results were obtained, the experiment was conducted again, using more controlled variables, including flavor of the gum, extraction incubation times, environmental exposure times, and time intervals between sample chewing and burial. The extraction methods described above were also used for this additional set of samples.

Based on the results obtained from both rounds of experiments, it was determined that the most effective method for DNA extraction from a forensically relevant chewing gum sample was the PCIA extraction method, rather than the PrepFiler® BTA™ or custom extraction methods also tested.

#### PrepFiler® BTA™, DNA Extraction, Chewing Gum



## Young Forensic Scientists Forum—2020

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### Y28 Using Loop-Mediated Isothermal Amplification (LAMP) to Identify At-Risk Species in the Field

Brooke Driscoll\*, Orchard Park, NY 14127; Nickolas P. Walker, BS, East Amherst, NY 14051; Jan E. Janecka, PhD, Duquesne University, Pittsburgh, PA 15282

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**Learning Overview:** After attending this presentation, attendees will better understand LAMP and its use as a field method for identifying species of interest for wildlife forensics.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a species identification technique for trafficked animal samples that can be performed directly in the field.

Species identification through DNA analysis is relevant to population genetics, species conservation, and wildlife forensics. Unfortunately, many species of interest are endemic to areas with little access to advanced scientific technology. This means that DNA testing must be outsourced to other areas or testing must wait until the researchers are in a different location. Additionally, the biological substrate for the species of interest may contain limited amounts of DNA. This pertains mainly to highly elusive species that can only be readily studied using scat and to trafficked samples such as teeth, claws, and scales. All of these substrates pose greater DNA processing difficulties when compared to the more traditional blood samples. To better aid in the research of endangered and at-risk species, a method is needed that can accurately identify species through unconventional biological substrates with minimal laboratory instrumentation.

A promising solution to this issue is LAMP. This amplification method has similar sensitivities to traditional Polymerase Chain Reaction (PCR) but can be done at isothermal temperatures. This eliminates the need for a thermocycler during the reaction. LAMP has also been studied with fluorescent, in-tube visualization methods that reduce the need for agarose gel. The entire process is relatively fast, requiring only a 30-minute incubation. Using these methods, it is possible that species could be identified in a field environment.

The main question to be answered by this research is: can a field-accessible kit be developed for non-invasive species identification using the LAMP method? Previous research has shown that isothermal amplification can be performed with minimal laboratory equipment, but a functioning kit has not been assembled that can accurately identify species in the field. The species of interest in this study is the snow leopard, which is highly elusive and difficult to study. A non-invasive sampling source for DNA is often scat, which poses difficulties for DNA analysis due to inhibitors. Creating a method based off of this substrate will allow the method to be more adaptable to other low-level DNA substrates.

The method is conducted primarily using a heat block, a fluorescent dye, components of the Lucigen® LavaLAMP™ kit, and an animal sample. The suspected snow leopard scat is collected in Mongolia and China, dried with a desiccant material, and shipped to the United States for testing. Approximately 100 of these scat samples will be rehydrated for testing. The DNA is tested by dipping Whatman® No. 1 filter paper into the scat, then into a lysis buffer, and then placed directly into the LavaLAMP™ amplification mix. Prior to incubation, calcein and manganese (II) chloride are added as fluorescent indicators. The samples are heated on a heat block to 70° Celsius for 30 minutes. Following amplification, the samples are immediately visualized under Ultraviolet (UV) light, with fluorescence being indicative of amplification. The expected results of this study are that the scat samples can be identified with high accuracy and reliability without the use of a thermocycler or agarose gel. Initial results have shown that the method is reliable with scat samples and can be visualized using the calcein fluorescence. Once further optimized, the method will be applied in China so that samples will not need to be sent to the United States for analysis. This kit will allow wildlife forensics and species conservation efforts to be conducted in resource-poor areas to accurately identify endangered and trafficked species.

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#### LAMP, Wildlife Forensics, Field





## Young Forensic Scientists Forum—2020

### Y29 FARO® Laser Scanner as a Tool for Bloodstain Pattern Analysts: Documentation of Bloodstains Enhanced by Luminol and BlueStar®

Tiffany Hogberg, BA\*, Syracuse University, Syracuse, NY 13210

**Learning Overview:** The goal of this presentation is to test the FARO® laser scanner's ability to capture chemiluminescence created by the application of luminol and BlueStar® to a dilution series of bloodstains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by beginning to define the capabilities of a 3D crime scene documentation system and testing if it can be advantageous for analysis as well as documentation.

**Hypothesis:** Due to the limitations of the FARO® Laser Scanner's equipment, it is predicted that the current methodology used to document luminol and BlueStar® reactions, photography, will be more accurate in documenting the chemiluminescence of the two reagents.

Luminol and BlueStar® have commonly been used at crime scenes to locate potential bloodstains.<sup>1</sup> Luminol is known to have a shorter chemiluminescence than BlueStar®, while BlueStar® is known to enhance potential bloodstains in locations that have more light exposure, such as outdoors scenes. Photography is the documentation for these methods of stain enhancement, with photographs being taken within seconds of luminol and BlueStar® application. There have been new developments in crime scene documentation due to the desire to capture the scene "as-is" for litigation; however, experimentation has not been done to test the capabilities of these new systems in documenting crime scenes that have been enhanced using reagents such as luminol and BlueStar®. This research will use the FARO® laser scanner to document bloodstain patterns enhanced with these reagents.

**Experimental Design/Materials and Methods:** This experiment is set up as four trials repeated three times. Two sets of bloodstains made from blood diluted at 1:1, 1:10, 1:50, 1:100, 1:500, and 1:1000 and will be created on two different surfaces, carpet and concrete, using 5mL of sheep's blood. The stains will be created and allowed to dry for two hours prior to their washing. The stains will be washed away using dish soap and water. The carpet and concrete will be allowed to dry for 24 hours prior to application of the reagents. One set of diluted bloodstains on the carpet will be enhanced using luminol and the other set using BlueStar®. The same procedure will be followed for the bloodstains on the concrete. Both the FARO® laser scanner and a Nikon® D7200 will be set up to record the reactions of each bloodstain. The documentation will be completed in a mostly dark environment.

The results for each stain will be evaluated to determine the sensitivity of the reagents, as well as the effectiveness of both means of documentation. The FARO® laser scanner is predicted to document the BlueStar® reactions better than the luminol reactions, as BlueStar® is known to have a longer chemiluminescence. The FARO® laser scanner takes longer to document the room because of the amount of data it is collecting. Scans roughly take five minutes to complete. However, because of the time required to scan a room using the FARO® laser scanner, it is predicted the documentation from the Nikon® D7200 will be the most effective in capturing the reactions. As for the sensitivity of each reagent, it is predicted that the BlueStar® will be more sensitive than the luminol to highly diluted bloodstains. This belief is based on known prior experiments in forensic literature of BlueStar® and luminol sensitivity.

#### Reference(s):

- <sup>1</sup> James, Stuart H., Paul E. Kish, T. Paulette Sutton. The Detection of Blood Using Luminol. In *Principles of Bloodstain Pattern Analysis: Theory and Practice*, [369-389]. CRC Press, 2005.

**Bloodstain, FARO® Laser Scanner, BlueStar®**



## Young Forensic Scientists Forum—2020

### Y30 Methamphetamine Confirmation Analysis After Controlled Vicks® VapoInhaler™ Injection Into Oral Fluid

Julia N. Canello\*, Pittsburgh, PA 15220; Stephanie J. Wetzel, PhD, Duquesne University, Pittsburgh, PA 15282; Frederick W. Fochtman, PhD, MolecularDx, LLC, Windber, PA 15963-1300

**Learning Overview:** The goal of this presentation is to further investigate false-positive assay testing for methamphetamine after a common nasal decongestant has been applied to neat oral fluid samples using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring enantiomer concentrations of methamphetamine after positive assay results using oral fluid, a matrix not commonly used for routine assay testing. This study will also allow attendees to understand the relationship between enantiomeric compounds, such as methamphetamine, and how their effects can be used for very different outcomes.

Methamphetamine, a Schedule II stimulant of the Amphetamine family, is currently the second most widely abused illicit drug in the country.<sup>1</sup> Methamphetamine is sought for its extensive psychoactive stimulant effects that include instant subtle psychosis, attention deficit, and decreased motor skill ability.<sup>2</sup> Two isomers of the drug, dextro- (D-) and levo- (L-), form its composition; however, each produce very different effects when active. For example, many over-the-counter nasal decongestants contain both isomers but only produce the vasoconstriction qualities of L-methamphetamine (L-MAMP). As a result, false positive assays for methamphetamine have consistently been an issue in the forensic community for years. Specifically, drug assay screenings have been known to read positive for methamphetamine use after Vicks® VapoInhaler™ (and other nasal decongestants) intake using various matrices, resulting in a false positive for the illegal substance D-Methamphetamine (D-MAMP). The unfortunate results propose an increased need for confirmatory methods after such occurrences. This research aims to identify false positive assay results for methamphetamine associated with Vicks® VapoInhaler™ injection into neat oral fluid samples.

Previous research has been conducted in order to recover D-MAMP after Vicks® intake using matrices such as urine and blood, but has only scarcely been investigated using oral fluid. Current research used a Molecular Devices SpectraMax® i3x Plate Reader to measure absorbances of oral fluid samples that were spiked with varying concentrations of Vicks® products. An Immunalysis™ Methamphetamine Assay Kit was used to test the amount of D-MAMP present in such samples at a range of 0-100ng/mL. The Limit Of Quantification (LOD) cutoff of this test was found to be 50ng/mL, a concentration that can prove comparable to workplace assay kits. Solid Phase Extraction (SPE) was used for samples that provided a positive assay to prepare for LC/MS/MS using a positive pressure manifold and Strata™-X strong cation Phenomenex® SPE cartridges. The quantity of D-MAMP was detected and confirmed using the Agilent® Technologies 6460 LC/Triple Quadrupole/MS operating in positive mode.

It has been hypothesized that the oral fluid samples that have been injected with higher concentrations of Vicks® products will produce false positive assay results for the illegal substance D-MAMP. Method validation for the SPE and LC/MS/MS parameters for extracting methamphetamine from varying oral fluid samples has proven to work successfully. Proof of concept using the SpectraMax® i3x has been completed using both positive control samples and spiked oral fluid samples with D-MAMP, L-MAMP, and D/L-MAMP standards, showing a subtle yet consistent decrease of absorbances in increasing D-MAMP concentrated samples.

Detecting exact concentrations of both D-MAMP and L-MAMP isomers in these Vicks® products can eventually lead to more specific immunoassays that will produce fewer false positives. The use of oral fluid can provide law enforcement and workplace testing facilities an easier collection method for drug screening. Nasal decongestants such as Vicks® VapoInhaler™ are used in many people's everyday lives across the nation, so it is vital that the forensic science community understand the process and work toward a solution to reduce false positive results.

#### Reference(s):

1. Marilyn A. Huestis, Edward J. Cone. Methamphetamine Disposition in Oral Fluid, Plasma, and Urine. *HHS Author Manuscripts*, 2007, doi:10.1196/annals.1384.038.
2. National Institute on Drug Abuse. 2019. *DrugFacts: Methamphetamine*.

#### Methamphetamine, LC/MS/MS, Drug Screening



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